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JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

VOLUME 49 — *SEPTEMBER 1960* — NUMBER 9

Investigation and Development of Protective Ointments III*

Adsorption Characteristics of Sarin from Solutions

By K. F. FINGER†, A. P. LEMBERGER, D. E. WURSTER, and T. HIGUCHI

The adsorption of sarin from *n*-heptane solutions by silica gels, alumina, bentonite, and certain carbonaceous adsorbents was studied. The results obtained have been interpreted according to the Langmuir adsorption isotherm. Of the various adsorbents tested, those of a silicaceous nature were found to be superior, exhibiting very strong adsorptive tendencies toward the fluorophosphate ester. A linear relationship was found to exist between the limiting adsorptive capacity and the specific surface area of the adsorbents. The average area occupied by a sarin molecule, furthermore, was found to be approximately 80 Å.^{2}, irrespective of the adsorbent, a value in reasonable agreement with that suggested by a molecular model. Both of these relationships indicate that sarin is adsorbed primarily as a unimolecular layer.

THESE INVESTIGATIONS were undertaken to provide basic information pertaining to the nature, intensity, and capacity of certain commercial materials to adsorb sarin.[1] Such information was desirable as a part of the study on protective ointments. It was felt that the information thus obtained would be useful in determining the relationship between the protective capacity of various filler materials and their adsorptive properties. The data, furthermore, were expected to prove useful in developing other protective measures against this and related agents.

This deals with the experimentally determined adsorption behavior of sarin on commercial adsorbents such as silica gels, alumina, bentonite, and certain charcoals from an organic solvent. A method of utilizing this data to predict adsorption behavior from other systems is also discussed.

* Recieved August 21, 1959, from the University of Wisconsin School of Pharmacy, Madison.

Presented to the Scientific Section, A. PH A., Cincinnati meeting, August 1959.

This research project was supported by the Directorate of Medical Research, U. S. Army Chemical Warfare Laboratories, Army Chemical Center, Md., under CmlC Contract No. DA18-108-Cml 2576.

† Present address: Charles F. Pfizer Co., Groton, Conn.

[1] Isopropoxymethylphenyl fluoride

EXPERIMENTAL

The amount of sarin removed from a solution by a solid adsorbent was determined by a relatively simple laboratory procedure which was contingent upon a state of equilibrium being established within the system. A weighed quantity of the adsorbent, approximately 0.1 Gm., was placed in a 25-mm. glass-stoppered weighing bottle and exactly 5 ml. of a known concentration of sarin in normal heptane was pipetted into the bottle. The suspension was then shaken at constant temperature for one hour[2] and then centrifuged. The supernatant liquid was analyzed for its sarin content by the Schoenemann method (2). The weight of sarin adsorbed by 1 Gm. of the adsorbent was calculated from the difference between the initial and equilibrium concentrations of sarin.

The adsorption isotherms were obtained by repeating the above procedure utilizing different initial concentrations of sarin in the solution. All determinations were done in duplicate at 25°. The following adsorbents were studied: silica gels Nos. 21, 63, 72, and 79, SiO_2 (Davison Chemical Co.); Santocel 54, Silica Aerogel (Monsanto Chemical Co.); Sylloid 244, SiO_2 (Davison Chemical

[2] Under the conditions of these experiments, it was previously established that equilibrium was attained within the one-hour period.

Co.); activated alumina (Aluminum Ore Co.); bentonite (Chattanooga Pharmacal Co.); Darco KB and S51, activated charcoals (Darco Division, Atlas Powder Co.); and wood charcoal (J. T. Baker Chemical Co.).

These adsorbents were selected on the basis of their applicability to ointment formulation and also because they were representative of a large number of commercial adsorbents now in use.

RESULTS AND DISCUSSION

Langmuir's adsorption equation may be stated as

$$x/m = k_1k_2c/(1 + k_1c) \qquad \text{(Eq. 1)}$$

where x/m is the weight of adsorbate adsorbed per unit mass of adsorbent, c is the equilibrium concentration of adsorbate, and k_1 and k_2 are constants. Brunauer has stated (1) that the constants of the Langmuir equation are neither arbitrary nor empirical but, rather, bear physical significance. The adsorption coefficient, k_1, is related to the force which binds the adsorbate molecules to the adsorbent surface and k_2 is the maximum amount of solute which will be adsorbed per unit mass of adsorbent.

A somewhat more useful form of the Langmuir equation is obtained by inverting Eq. 1 and multiplying through by c, thus

$$\frac{c}{x/m} = \frac{1}{k_1k_2} + \frac{c}{k_2} \qquad \text{(Eq. 2)}$$

A plot of $c/(x/m)$ *versus* c is linear with a slope of $1/k_2$ and an intercept of $1/k_1k_2$. The adsorption isotherms for several silicaceous adsorbents, alumina, bentonite, and three types of carbonaceous adsorbents were plotted according to Eq. 2 and a sample plot is given in Fig. 1.

It was evident from these plots that with all the adsorbents except Darco S51 and wood charcoal, adsorption of sarin obeyed the linear requirements for $c/(x/m)$ *vs.* c plots. A plot of x/m *vs.* c for Darco S51 (Fig. 2) yielded a sigmoid curve characteristic of the Type V isotherm described by Brunauer (3). Although a further study of this system would be of theoretical significance, the extremely low capacity shown by this adsorbent at low sarin concentrations precludes its consideration as a filler material. A similar plot of x/m *vs.* c was made for wood charcoal (Fig. 3) since the adsorption of sarin was so limited over the concentration range employed that it could not be characterized.

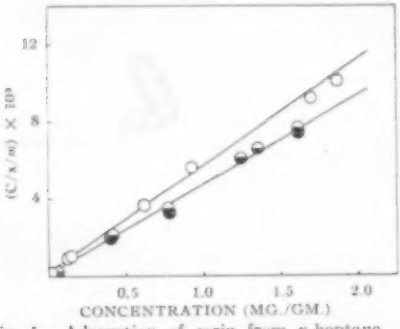

Fig. 1.—Adsorption of sarin from *n*-heptane. ○, Silica gel No. 21; ●, silica gel No. 63.

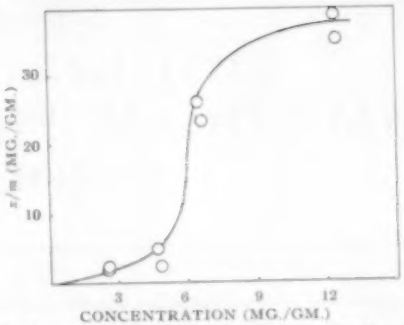

Fig. 2.—The adsorption of sarin from *n*-heptane by Darco S-51.

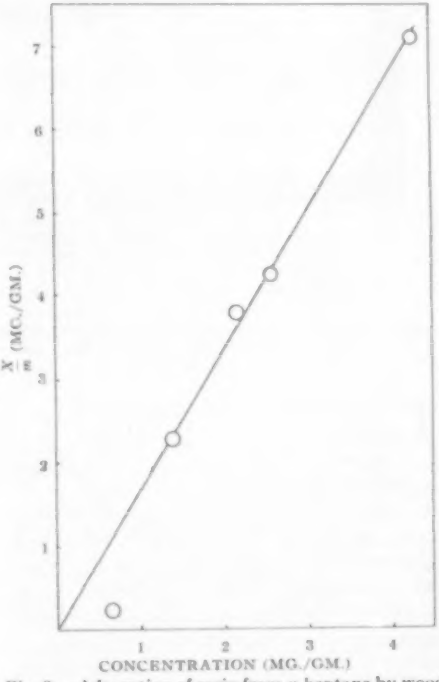

Fig. 3.—Adsorption of sarin from *n*-heptane by wood charcoal.

Brunauer has stated that a linear relationship between $c/(x/m)$ and c alone is not sufficient evidence that the system follows Langmuir's type adsorption. It is also necessary that the values obtained for the adsorption coefficient and the limiting adsorptive capacity be within reasonable limits (1). Thus, the limiting adsorptive capacity, k_2, should be proportional to the surface area of the adsorbent if the hypothesis of unimolecular adsorp-

tion is valid and, furthermore, should yield a reasonable value of the area occupied by each adsorbed molecule under close order packing. The adsorption coefficient, k_1, is dependent upon the nature of the surface and, consequently, should be nearly constant for a homologous series of adsorbents.

The values for the limiting adsorptive capacity, k_2, for the various adsorbents obtained from the Langmuir plots are listed in Table I. Comparison of these data indicates that the silicaceous adsorbents are superior to the other agents tested as adsorbents for sarin.

TABLE I.—PHYSICAL CONSTANTS FOR THE ADSORPTION OF SARIN FROM *n*-HEPTANE

Absorbent	Surface Area, M^2/Gm.	$k_1 \times 10^{3}$ [a] Gm./mcg.	$k_2 \times 10^{-3}$ [b] mg./Gm.
Santocel 54	175	...	0.38
Sylloid 244	292	6.8	1.20
Silica gel No. 72	300	4.8	0.95
Silica gel No. 79	400	4.9	1.06
Silica gel No. 21	600	6.5	1.84
Silica gel No. 63	800	8.6	2.13
Alumina F-1	200	...	0.58
Bentonite	470	0.18	1.23
Darco KB	600–1,200	0.07	1.49

[a] Adsorption coefficient. [b] Limiting adsorptive capacity.

The determination of the k_1 values presented a somewhat greater problem because of the very strong affinity of the phosphate esters for the majority of the adsorbents. Indeed, only approximate evaluation could be made. The usual method of calculating the adsorption coefficients is to determine them from the intercept of the straight line plot of the Langmuir equation. This procedure could not be used effectively in this study because of the close proximity of the interception point with the origin. Instead, an alternate method was employed.

It is evident from the linear Langmuir equation that the adsorption coefficient is described by the following relationship

$$k_1 = \frac{1}{c} \frac{(x/m/k_2)}{1 - (x/m/k_2)} \qquad \text{(Eq. 3)}$$

where the quantity $(x/m/k_2)$ is the fraction of the adsorbent's surface occupied by adsorbate molecules and, therefore, $(1 - x/m/k_2)$ is the fraction left unoccupied. Designating the fraction occupied as F_c and substituting into Eq. 3, we find

$$k_1 = \frac{1}{c} \frac{F_c}{1 - F_c} \qquad \text{(Eq. 3a)}$$

It is obvious from the above equation that k_1 is numerically equal to $1/c$ when the ratio $F_c/1 - F_c$ is unity.

The procedure followed for the evaluation of the k_1 values, therefore, was to plot $F_c/1 - F_c$ against the corresponding equilibrium concentrations of the adsorbate as shown in Figs. 4 and 5 and to determine graphically, the concentration when $F_c/1 - F_c$ was unity. It should be emphasized here that the method required the data to be selected in the range of equilibrium concentrations such that a substantial portion of the surface of the adsorbent

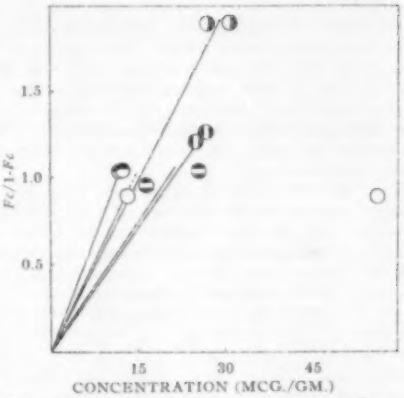

Fig. 4.—Plot to determine the adsorption coefficients of the silicaceous adsorbents. ◑, Silica gel No. 72; ◓ silica gel no. 79, ◓, silica gel no. 21; ◑, silica gel no. 63; and ○, Sylloid 244.

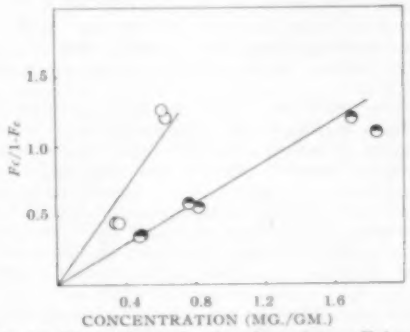

Fig. 5.—Plot to determine the adsorption coefficients. ○, Bentonite; ◓, Darco KB.

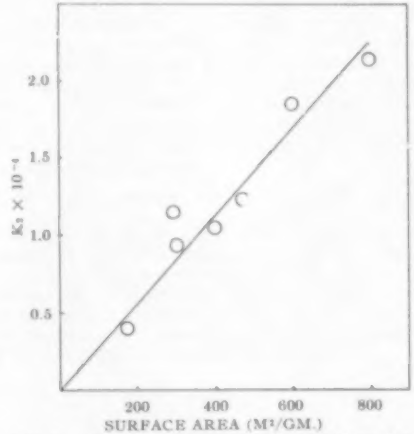

Fig. 6.—Relationship between the limiting adsorptive capacity and the specific surface area of the various adsorbents.

was still uncovered. For adsorbents such as the silica gels, which approach saturation even in very low concentration ranges, considerable error was to be expected. It is evident from the data used in Figs. 4 and 5 that only approximate values of the adsorption coefficient can be obtained for the silica gels. These are listed in Table I. The values for bentonite and Darco KB are relatively more dependable.

The question whether these data support the original assumption of formation of a unimolecular layer on the adsorbent surface can be tested by determining the relationship between the available specific surface area of these materials and their limiting adsorption capacity toward sarin.

If the adsorption is unimolecular, the total number of sarin molecules that can be adsorbed will be linearly dependent upon the area of the surface that is available for adsorption. Figure 6 shows the relationship that exists in the systems. The specific surface areas used were those provided by the source of each material. From the slope of this line the average area occupied by an adsorbed molecule can be calculated. The average area calculated for a sarin molecule from the slope of the line was found to be approximately 80 $Å.^2$. To check this value, a scale model of the sarin molecule was constructed and its molecular area roughly determined. The two values were in reasonable agreement.

The adsorption coefficients listed in Table I show a dependence on the chemical nature of the surface. For instance, within a homologous series such as the silica gels, the coefficient assumes an approximately constant value. The surface of the silica gels, alumina, Santocel 54, and Syloid 244 is highly polar in nature while that of the charcoals is relatively nonpolar. Inasmuch as the adsorption coefficient is an index of the primary binding force, the values obtained for the more polar adsorbents should be larger than those obtained for the relatively nonpolar adsorbents. That this relationship holds can be seen by comparing the values of k_1 obtained for the silica gels with those obtained for the charcoals.

While the classification of adsorbents with respect to the polarity of their surface must remain relative, it nevertheless yields some basis for the selection of an adsorbent for a specific adsorbate.

Knowledge of the adsorption characteristics of the penetrant, such as determined in the present study, can be useful information in the formulation of protective ointments. The limiting adsorptive capacities, k_2's, of the various adsorbents would necessarily be independent of the solvent employed if solvent adsorbent interactions are negligible. Thus, proper selection of an ointment base would provide a medium for maximum adsorption of the noxious material.

In contrast, the adsorption coefficient, k_1, is highly dependent upon the solvent from which adsorption occurs. However, in the absence of any strong interaction between solvent and adsorbent, it is possible to obtain a relatively simple relationship between k_1 values for the adsorption of an agent from any two solvent systems by a given adsorbent if the effective partition coefficient of the agent between the two liquids is known. By effective partition coefficient is meant here, the ratio of concentration of the agent present in each solvent when brought into equilibrium with a fixed vapor concentration of the agent. To illustrate, if the partition coefficient of the agent is such that a solvent is favored by a factor of three over the reference solvent, the k_1 value of the agent on any of the adsorbents will be one-third that in the reference solvent. Since partition data are readily available, calculations of this type can be carried out for many systems.

It is possible, then, to select a vehicle for the adsorbent in which the adsorption coefficient for the penetrating agent is such that significant retardation of permeation at toxic concentration levels of agent occurs.

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Investigation and Development of Protective Ointments IV*

The Influence of Active Fillers on the Permeability of Semisolids

By K. F. FINGER†, A. P. LEMBERGER, T. HIGUCHI, L. W. BUSSE, and D. E. WURSTER

The permeability of protective barriers containing adsorbent type fillers has been studied. It has been shown that the time for the nonstationary state permeation of these barriers can be significantly increased by the incorporation of small quantities of an active filler into the ointment base. The theoretical considerations of permeation through filled ointments have been presented with particular emphasis placed on the theory pertaining to barriers containing a filler with a high affinity for the penetrant. An equation relating the lag time to the concentration and maximum adsorptive capacity of this type filler and to the initial concentration of the chemical agent has been derived and verified by experimental procedures.

THE THEORETICAL and experimental investigations of the possible values of active fillers in improving the degree of protection afforded by protective creams and ointments are presented in this report. The term active filler, as used here, includes all fillers which, when incorporated into an ointment or cream base, substantially reduce the chemical or physiological activity of toxic agents by preventing or diminishing the transport of the agent in an active form through the protective barrier.

As pointed out earlier by Higuchi (1), the protective value of creams and ointments during the early stages of penetration does not always correlate with their effectiveness during the later phases. This is especially true with active fillers, the effectiveness of these additives being limited, for all practical purposes, to the initial, nonstationary phase of permeation. For this reason, the present treatment is limited primarily to this initial or nonstationary phase.

THEORY

The manner in which active fillers manifest their protective action is highly dependent on the way the filler itself reacts with the penetrating agent. It is convenient then for the present purposes to assume two limiting cases for this interaction:

Case I.—The thermodynamic activity of the chemical agent in equilibrium with the filler material is considered to be directly proportional to its relative concentration. Thus, if a linear partition isotherm exists within the system, this condition is satisfied, and

$$a \propto x/m$$

where a is the activity of the agent and x/m is the amount of the agent reacted with the filler. This situation would arise, for example, if the filler material was a liquid with a relatively strong affinity for the penetrant. A fair approximation of this case also exists for the adsorbent type filler which possesses a relatively low k_1 value as derived from the Langmuir relationship (2).

Case II.—The thermodynamic activity of the penetrant in equilibrium with the filler is considered to be negligibly small until a critical amount of the agent has been taken up. Beyond this point, the activity increases directly with the amount of agent added. This type of behavior exists, in the case of a strongly adsorptive filler which is characterized by a relatively high k_1 value. The filler possessing a high k_1 value will reach a point of saturation at very low equilibrium concentrations of the penetrant.

The mathematical relationships governing the nonstationary state permeation of filled systems considered under case I have been treated in part by Higuchi (1). When a linear partition isotherm exists within a diffusional system, Fick's second law must be modified to account for the amount of penetrant entering the filler phase. The modified equation may be written

$$dc/dt = (D/V_c + KV_f)(d^2c/dx^2) \quad \text{(Eq. 1)}$$

where V_c and V_f are the volume fractions of the continuous and filler phases, respectively, and K is the distribution coefficient of the penetrant between the two phases. The movement of the concentration front across the barrier under a driving force of a changing concentration gradient is then described by the following equation

$$C = C_0\left[1 - \frac{2}{\sqrt{\pi}}\int_0^{x/2\sqrt{dt/V_c+KV_f}} e^{-y^2}dy\right] \quad \text{(Eq. 2)}$$

where C_0 is the initial concentration of the penetrant in the barrier.

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† Present address: Charles F. Pfizer Co., Groton, Conn.

It is evident from the above equation that, for a system obeying the conditions given under case I, the lag time will be considerably greater than that obtained for a homogeneous system. Higuchi has stated that this ratio will be equal to $(V_c + KV_f)$.

In a homogeneous system, that is, one that does not contain a filler material, the lag time, or time required for the concentration front to move through the barrier, is related to the thickness of the barrier and the diffusion constant by the relationship first derived by Daynes (3) and developed by Barrer (4)

$$L = \frac{x^2}{6D} \qquad \text{(Eq. 3)}$$

where L is the lag time, x is the thickness, and D is the diffusion constant. Barrer and others have used this relationship to evaluate diffusion constants from permeability data. Thus for a filled system of case I type

$$L = (V_c + KV_f)\frac{x^2}{6D}$$

A similar relationship may be derived for systems containing a filler material which obey the conditions proposed under case II, above. If a linear partition isotherm does not exist within the system, neither Fick's second law nor the modification of it given for case I system is valid; therefore, special boundary conditions must be assumed.

As the penetrant enters the barrier containing a filler with high affinity for the agent, the penetrant is effectively removed from solution by the filler. As a result of the relatively high affinity of the filler for the penetrant, the filler must be saturated before the concentration front is free to progress through the barrier. In the case of the adsorbent type fillers, the above condition implies the maximum adsorptive capacity of the filler must be attained.

Thus, for the experimental system described later in this report, consisting of a barrier membrane whose one surface is brought into equilibrium with the vapor of the penetrating chemical, the rate of movement of the agent through the barrier can be related to other variables in the system in the following manner.

For such a system containing a case II type filler, the concentration gradient of the penetrating chemical in the external phase would approximate that shown in Fig. 1, where the concentration of the penetrating chemical agent within the external phase has been plotted against the distance. For the idealized case where essentially all of the agent entering into the system is found in the filler phase, the gradient, it can readily be shown, will be strictly linear.

It is evident that the total amount of material which has entered such a barrier is the sum of the material found in the external phase and that taken up by the filler. Since the average concentration of the agent up to the point of maximum penetration is $^1/_2\ C_0$, where C_0 is the concentration of penetrant in the surface layer exposed to the vapor of the penetrating chemical, the amount in the external phase is equal to $^1/_2\ C_0xH$, where x is the depth of penetration and H is the cross sectional area of the barrier.

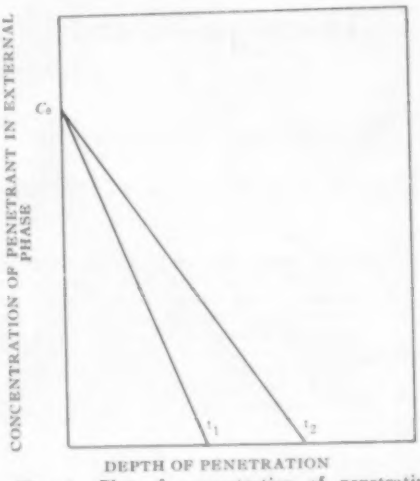

Fig. 1.—Plot of concentration of penetrating chemical in external phase for idealized case II at times t_1 and t_2. C_0 is the concentration of penetrant in surface layer exposed to the vapor of penetrating chemical.

The amount of material taken up by the filler up to this point, on the basis of previous reasoning, is equal to AxH, where A is the amount of penetrant adsorbed by the filler per unit volume of the barrier material. The total amount of penetrant necessary to permit a small additional penetration, dm, would then be

$$dm = {}^1/_2\ C_0\ Hdx + AHdx \qquad \text{(Eq. 4)}$$

But since the amount of material crossing a plane of unit area per unit time, Q,

$$Q = (dm/dt) = -DH(dc/dx)$$

the equation may then be written

$$\frac{{}^1/_2\ C_0Hdx + AHdx}{Hdt} = -D(dc/dx) \qquad \text{(Eq. 5)}$$

If the concentration gradient is assumed to be linear over the distance penetrated by the chemical, it is then valid to express the concentration gradient as

$$dc/dx = C_0/x$$

This then leads to the equation

$$\frac{{}^1/_2\ C_0Hdx + AHdx}{Hdt} = -D(C_0/x) \qquad \text{(Eq. 6)}$$

which may be reduced and rearranged to give

$$\frac{({}^1/_2\ C_0 + A)\,xdx}{C_0} = -D\,dt \qquad \text{(Eq. 7)}$$

Or, expressed as an integral equation, it becomes

$$\int_0^x \frac{({}^1/_2\ C_0 + A)xdx}{C_0} = -D\int_0^t dt \qquad \text{(Eq. 8)}$$

The resulting integrated equation then is

$$L = \frac{x^2}{4D} + \frac{Ax^2}{2C_0D} \qquad \text{(Eq. 9)}$$

where L represents the time required for the concentration front to move through the barrier, i. e., the lag time.

The amount of penetrant adsorbed by an adsorbent type filler is a function of its maximum adsorptive capacity and thus the quantity A may be replaced by $C_f k_2$, where C_f is the concentration of the filler and k_2 is the maximum adsorptive capacity of the filler as determined from adsorption experiments (2). The amount of penetrating chemical entering the barrier is governed by the distribution coefficient of the agent between the solvent and the barrier material; then C_0 is equal to KC, where K is the distribution coefficient and C is the initial concentration of the agent in the solvent. Thus, the final equation becomes

$$L = \frac{x^2}{4D} + \frac{C_f k_2 x^2}{2KCD} \qquad \text{(Eq. 10)}$$

The usefulness of Eq. 10 lies in the fact that the contribution of an adsorptive type filler can be predicted from adsorption data without recourse to the more difficultly obtained permeability data. In the case of the filler being a chemically active detoxicant, a similar equation would result.

It is interesting to note that Eq. 10 predicts a lag time equal to $x^2/4D$ for the extreme case where none of the penetrant adsorbed, a value which compares with the theoretical lag time for this case, $x^2/6D$. Thus, it would seem that Eq. 10 can be expected to give reasonably useful values for lag time even for those cases which deviate appreciably from case II.

EXPERIMENTAL STUDIES

The influence of the active fillers on the nonstationary permeability of protective barriers was determined by measuring the amount of sarin permeating the barriers as a function of time. The data was then plotted and the lag time for the system determined by extrapolating the straight line portion of the permeability curve to the time axis. In each phase of this work, white petrolatum was used as a control and was also used as the ointment base into which the fillers were incorporated.

Apparatus.—The apparatus used in this study was the barrier cell apparatus previously described (5). The bronze washers used were 0.043 cm. thick and possessed an internal diameter of 2.22 cm.

Preparation of the Ointments.—A weighed quantity of the melted ointment base, white petrolatum, was placed in a 50-ml. beaker and a weighed quantity of the filler sufficient to produce the desired strength ointment (w/w) was added. The mixture was then stirred to disperse the filler throughout the ointment base. The molten mass was placed in a Bell jar and the system evacuated for at least five minutes to remove any dissolved or adsorbed gases. A second stirring was then initiated and continued until the ointment had congealed. Immediately before the barriers were prepared, the ointment was again evacuated to remove any gases entering the system as a result of the second stirring.

Preparation of the Barriers.—The barriers were prepared and mounted in the apparatus in the same manner as previously described (5).

Experimental Procedure.—The penetrant utilized in this study was fluorophosphate ester, sarin, dissolved in Amoil-S.[1] After the barrier cell apparatus assembly was completed, exactly 1.5 ml. of the penetrant solution was placed in one of the glass chambers and exactly 1.5 ml. of the pure solvent was placed in the other chamber, i. e., the receiving chamber. The assembly was then placed in a constant temperature bath maintained at 25° and the greased stopcocks were fitted into the glass chambers. Gentle shaking was maintained throughout the experiment by means of a shaking apparatus attached to the bath.

At definite time intervals, 0.1 ml. of the solution in the receiving chamber was removed with a pipet. The sample was placed in a tared, 25-mm. weighing bottle and the weight of the sample determined. The amount of sarin contained in each sample was determined by the Schoenemann method (6) utilizing a Klett-Summerson colorimeter.

The amount of sarin contained in the sample was converted to mcg./Gm. and this quantity was plotted against the time[2] in hours. The lag time for the system was obtained by extrapolating the straight line portion of the permeability curve to the time axis, the point of intersection being the lag time.

EXPERIMENTAL RESULTS

It has been shown (4) that the lag time is a measure of the rate at which the penetrant molecules diffuse through the barrier material until the concentration front reaches the boundary of the barrier and a pseudo-equilibrium state has been attained. The lag time is therefore a characteristic of the barrier system and may be used as a measure of the protective quality of the system. As a general rule, the longer this lag time, the greater the protective quality of the barrier.

In the permeability curves shown in Figs. 2, 3, 5, and 6, the concentration of sarin permeating the barrier, expressed as mcg./Gm. of solution, is plotted against the time, in hours. The lag time for the particular system may then be obtained by extrapolating the straight line portion of the permeability curve back to the time axis.

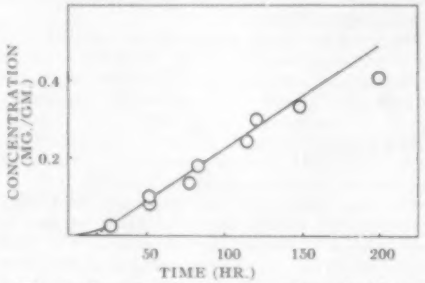

Fig. 2.—Lag time for the permeation of white petrolatum barriers by sarin. C = 45.0 mg./Gm.

[1] n-Amyl sebacate, Central Scientific Co.

[2] It was necessary to introduce a correction for the change of volume of solvent due to the withdrawal of samples. This correction was conveniently made by multiplying the time of sampling by the ratio of the initial volume of solvent to the actual volume remaining after the previous sample had been withdrawn.

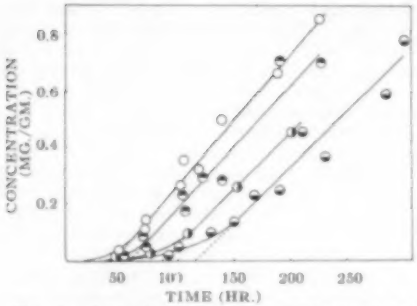

Fig. 3.—Lag time for the permeation of white petrolatum barriers containing 3% w/w of various silicaceous adsorbents by sarin. C = 45.0 mg./Gm.; ○, silica gel No. 72; ◓, silica gel No. 79; ◑, silica gel No. 21; ◒, silica gel No. 63.

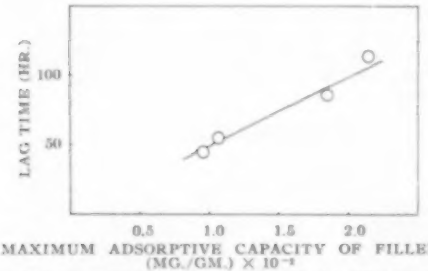

Fig. 4.—Lag time for permeation of a filled ointment barrier as a function of the maximum adsorptive capacity of the filler.

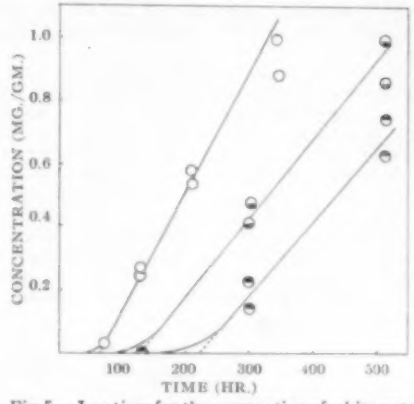

Fig 5.—Lag time for the permeation of white petrolatum barriers containing various concentrations (w/w) of silica gel No. 72 by sarin. C = 10.0 mg./Gm.; ○, 5%; ◒, 10% ◓, 15%.

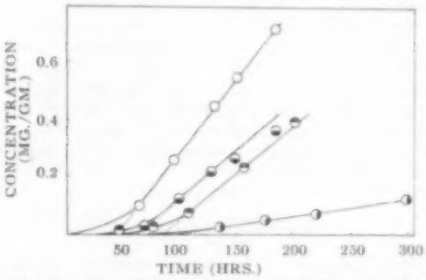

Fig. 6.—Lag time for the permeation of white petrolatum barriers containing 3% w/w silica gel No. 21 utilizing various concentrations of sarin in Amoil-S. ○, C = 87.3 mg./Gm.; ◒, C = 70.3 mg./Gm.; ◓, C = 45.0 mg./Gm.; ◑, C = 23.2 mg./Gm.

In Fig. 2, the permeability of white petrolatum barriers without a filler is shown. It is evident from the graph that the initial breakthrough of the penetrant occurs at approximately seventeen and one-half hours, or, applying the general rule stated above, the maximum degree of protection afforded by a white petrolatum barrier 0.043-cm. thick would be extended over a period of approximately seventeen and one-half hours.

While the maximum degree of protection afforded by a white petrolatum barrier is of the order of seventeen and one-half hours, the incorporation of as little as 3% (w/w) of an active filler produces a significant increase in the lag time for the system. This is evident in Fig. 3, where the permeability of white petrolatum barriers containing 3% (w/w) of the various silicaceous adsorbents is shown.

The lag times for the protective ointments containing the various silicaceous fillers are seen to vary with the specific surface area of the filler employed. Thus, lag times ranging from forty-five to one hundred and fifteen hours are observed for the 3% ointments. The maximum lag time, one hundred and fifteen hours, was obtained by using silica gel 63, a silicaceous adsorbent possessing a specific surface area of 800 M^2/Gm. This increase in lag time over that of white petrolatum represents an approximate sevenfold increase in the protective quality of the barrier. Proportionately lower protective indexes are observed for other silicaceous adsorbents possessing lower specific surface areas.

It has been shown that the maximum adsorptive capacity of a filler is linearly related to its specific surface area (2), that is, the maximum amount of penetrant that will be adsorbed by a filler is dependent upon the amount of surface available for adsorption. Equation 10, derived above, predicts a dependence of the lag time on the maximum adsorptive capacity of the filler. This relationship is shown in Fig. 4. The values for the maximum adsorptive capacity were obtained from adsorption studies previously completed in these laboratories (2) and the lag time values were obtained from Fig. 3.

This relationship between the lag time and the maximum adsorptive capacity is to be expected only in the event that the filler possesses a relatively high affinity for the penetrant. The relative affinity of the adsorbent for the penetrant is readily obtained from adsorption studies. The fact that the proportionality between the lag time and the maximum adsorptive capacity was found to exist

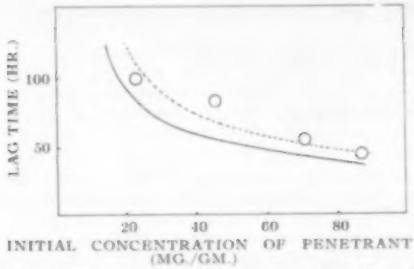

Fig. 7.—Lag time for the permeation of white petrolatum barriers containing 3% (w/w) silica gel No. 21 as a function of the initial concentration of penetrant. Solid line equals theoretical curve.

is, in part, verification for the basic assumption of complete saturation of the filler occurring during the nonstationary phase. Further indication that this assumption is valid may be observed in the rather sharp inflection in the permeability curves at the breakthrough point. If a linear partition isotherm existed in the system, a more gradual change in slope would be expected.

The complete saturation of the filler during the nonstationary phase of permeation has an important practical application to protective ointment formulation. If, for example, an ointment base is employed that has a very low affinity for the penetrant, then only a very small amount of the penetrant would be dissolved in the barrier. Inasmuch as a highly active filler must be saturated before the concentration front is free to progress through the barrier and the initial concentration of penetrant is very low, the incorporation of an active filler into such an ointment base would result in an almost indefinite lag time for the system.

While it is advisable to utilize a material possessing a maximum adsorptive capacity as a filler in protective ointments, a similar result may be obtained by increasing the relative concentration of the filler within the ointment. The mechanism of action is identical with that given above. An increase in concentration provides an increased surface area for adsorption and, as a result, the total amount of penetrant that can be adsorbed is increased. The relationship between lag time and the percentage filler is shown in Fig. 5, where the permeability of white petrolatum barriers containing various concentrations of silica gel No. 72 is shown.

Unfortunately there is a limitation to the amount of filler which can be incorporated into an ointment base since it is possible to incorporate so much filler into an ointment base that cracks or crevices are created which allow the penetrant to travel at an increased rate. This discontinuity in the protective barrier destroys any protective quality imparted to the ointment by the filler.

The initial concentration of penetrant in contact with the barrier is also important in influencing the lag time for the system, as is indicated in Eq. 10, where an inverse dependence of the lag time on the initial concentration is predicted. To substantiate the dependence of the lag time on the initial concentration, experimental determinations of the lag time were made utilizing different concentrations of sarin in Amoil-S as the penetrant. The data for these experiments are plotted in Fig. 6. It is evident from Fig. 6 that an increase in the starting concentration results in a decreased lag time.

It is evident from Eq. 10 that as C_0 becomes very large, the second term loses significance and the lag time approaches a limiting value, namely, $x^2/4D$. On the other hand, as C_0 approaches zero, the first term becomes insignificant and the lag time approaches an infinite value.

The agreement between the theoretical and experimental lag times over the range of concentrations employed in this work is shown in Fig. 7. In Fig. 7 the theoretical lag times, as calculated from Eq. 10, appear as a solid line while the experimental values appear as circles, connected by a dotted line. The shape of the experimental curve as compared with the theoretical illustrates a satisfactory agreement between the two.

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The Adsorption of Dodecylbenzenesulfonate and Hexachlorophene on the Skin*

By GERALD M. COMPEAU

A technique is presented for determining the adsorptive capacity of human skin and the skin of laboratory animals for dodecylbenzenesulfonate and hexachlorophene. The influence of time, pH, and concentration on the adsorption is studied and a mechanism for the adsorption and antibacterial activity of these compounds is postulated.

IT HAS LONG been known that a reaction takes place between anionic surfactants and proteins. The studies of Anson (1), Putnam (2), Putnam and Neurath (3), Valko (4), and others have considered the effect of lauryl sulfate, secondary alkyl sulfate, and dodecylbenzenesulfonate on substrates such as egg albumin, wool, casein, and horse serum albumin.

In recent years, as the incidence of skin irritation (5, 6) has paralleled the increased production and use of synthetic detergents, dermatologists have been concerned with the effect of these compounds on the intact human skin, largely (aside from the gross pathology) from the viewpoint of skin protein denaturation and the accompanying liberation of sulfhydryl groups from the keratin (7, 8, 9). While the older literature stressed the defatting action of the synthetic detergents, their chemical reactivity is now being given more attention.

Although hexachlorophene is almost exclusively used in products formulated to be applied to human skin, its adsorption on the skin surface has received little study. The rationale of its use is based on the fact that continued application of hexachlorophene-containing products reduces the number of viable bacteria which are removable from the skin with Ivory soap by test procedures such as the serial basin technique of Price (10) or modifications thereof (11). Fahlberg, Swan, and Seastone (12) ether-extracted thumbs after the use of hexachlorophene soaps and indirectly estimated the hexachlorophene by a bacteriological assay method.

EXPERIMENTAL PROCEDURES AND RESULTS

Materials.—The hexachlorophene used was the commercial material described in U. S. P. XV. The dodecylbenzenesulfonate was the triethanolamine salt of alkyl (kerosene) benzenesulfonic acid available as Nacconol 60S.[1] This contains 52% alkyl benzenesulfonate, 8% triethanolamine sulfate, and 40% water. It will be referred to as DBSA.

Determination of Hexachlorophene and DBSA on the Skin.—The method is based on the ultraviolet absorption spectra (Fig. 1) of these compounds in a solvent which is anhydrous methanol 0.015 *M* in phosphoric acid (acid methanol). The hexachlorophene is determined at 298 mμ and the DBSA at 262.5 mμ, correcting for the hexachlorophene found. The determination of the ultraviolet absorption spectra (in this solvent) of many purified dodecylbenzenesulfonates, whether based on kerylbenzene or tetrapropylbenzene alkylates, shows the presence of fine structure in the beta region of 255–270 mμ with a maximum at 262.5 mμ. Reid, Alston, and Young (13) find a $\lambda_{max.}$ very close to this value for many proprietary products using water as a solvent.

For determining the adsorption levels on the skin, an extraction technique is used which utilizes a glass-stoppered test tube of known cross-sectional area. To determine the rate of adsorption of hexachlorophene and DBSA as a scrub is carried out, the following procedure is used: obtain a blank value by scrubbing the hands with a brush for several minutes with Ivory soap. With 10 cc. of acid methanol in a test tube (15 × 150 mm., TS 16), extract a dried area of the skin 30 times by inverting the test tube over the skin surface. Scrub for two minutes with the test product and again extract the skin. Continue the scrubs and extractions for the period required and determine the absorbance of the acid methanol extracts at 262.5 mμ and at 298 mμ using standardized silica cells of 1.000 cm. (±0.5%) light path.

Calculations.—At 298 mμ the absorption of DBSA is negligible (Fig. 1); thus the absorbance at this wavelength can be taken as a direct measure of hexachlorophene: $K = A/bc$, where b = 1 cm. and c = Gm./L.

Since for hexachlorophene K_{298} = 14.9, and the cell is 1 cm., $14.9 = A_{298}/c$; and changing from Gm. per liter to mcg. per 10 ml.: mcg. per 10 ml. = $A_{298} \times 10^6/14.9 \times 10^3$.

As the 10 ml. contains the hexachlorophene extracted from the cross-sectional area of the test tube (2.12 sq. cm.), hexachlorophene, mcg./sq. cm. = $A_{298} \times 10^4/14.9 \times 2.12$.

With the hexachlorophene now being known, the DBSA is determined at 262.5 mμ, correcting for the absorbance of the hexachlorophene at that wavelength. This correction factor is the ratio $K_{262.5}/K_{298}$ for hexachlorophene, which is 0.0926.

Then, since $K_{262.5}$ for DBSA = 0.487,[2] DBSA

* Received December 31, 1959, from the Research Laboratories, Lehn & Fink Products Corp., Bloomfield, N. J.

[1] Allied Chemical Corp., Buffalo, N. Y.

[2] While this value is for Nacconol 60S, the determination may be made for other alkyl benzenesulfonates if the $K_{262.5}$ value is determined for the compound. While all commercial sulfonates contain small amounts of homologs of the dodecylbenzenesulfonate usually considered, the average absorption obtained gives satisfactory values.

mcg./sq. cm. = $A_{280.5} - (A_{256} \times 0.0926) \times 10^4 / 0.487 \times 2.12$.

It has been found that the rate of extraction of the hexachlorophene and DBSA from the skin is such that no significant increase in recovery is obtained beyond 15 extractions and that the 30 extractions used is adequate and requires less than two minutes to carry out. Studies of blank (Ivory) skin extractions show a value for hexachlorophene of about 0.5 mcg./cm.^{2} and with the lapse of time this value does not change appreciably. A usual blank value for DBSA is 20 mcg./cm.^{2} and this value will increase slowly with time. The ultraviolet absorption spectra of the blank extracts indicate that this increase in absorption in the DBSA range may be due to the extraction from the skin of tyrosine-containing proteins (14).

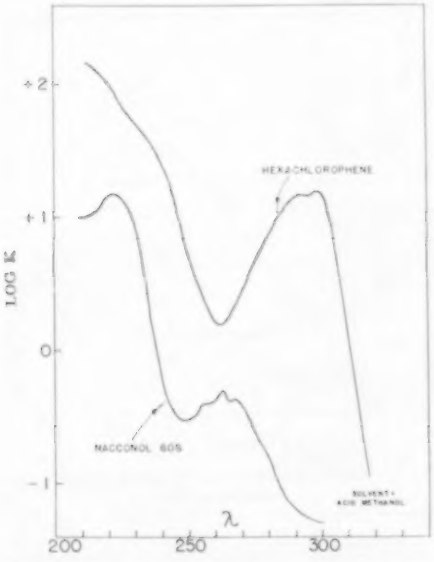

Fig. 1.—Ultraviolet absorption spectra of hexachlorophene and Nacconol 60S.

Efficiency of the Extraction Technique.—To estimate the completeness of extraction of the hexachlorophene and DBSA after absorption on a substrate, a scrub series was run using a swatch of undyed Botany worsted wool. This was scrubbed with Ivory soap (blank) and then with scrub No. 1681 for five × two minutes. As the scrubs progressed, the wool was extracted as usual. Although an exact comparison cannot be made since the actual area of the wool fibers is not known, the results with the wool are shown in Table I together with the results on the human palm.

TABLE I.—ADSORPTIVE CAPACITY OF WOOL

Substrate	Mcg. Adsorbed/Sq. Cm. Substrate: Hexachlorophene	DBSA
Wool (26 mg.)	14	135
Human palm	24	570

It is interesting to note that under these conditions the wool has taken up approximately 24% by weight of DBSA calculated as the dry Na salt, and 5% by weight of hexachlorophene. The DBSA value is of the magnitude found by Neville and Jeanson (15) for lauryl sulfate when concentration differences are allowed for. Since the wool showed an appreciable adsorption of both compounds, an effort was made to gauge the completeness of removal of the adsorbed materials. After the twelve-minute (cumulative) scrub the wool was sampled, chopped up, and refluxed with acid methanol (i. e., exhaustive extraction) along with an Ivory soap-scrubbed piece as a blank. The results are shown in Table II.

TABLE II.—RECOVERY OF ADSORBED COMPOUNDS FROM WOOL AFTER EXHAUSTIVE EXTRACTION

Process	Mcg./Sq. Cm.: Hexachlorophene	DBSA
Usual 30 extractions	15	174
Exhaustive extraction	17	270

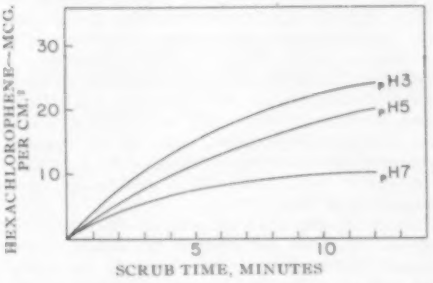

Fig. 2.—Effect of pH on the adsorption of hexachlorophene on the skin.

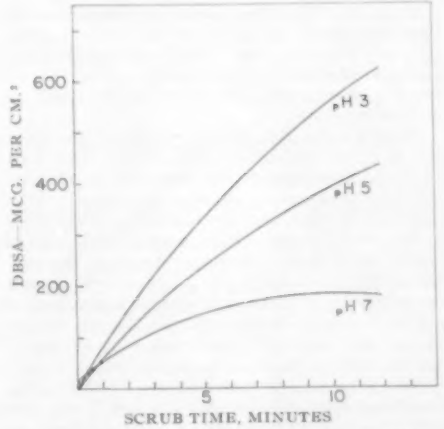

Fig. 3.—Effect of pH on the adsorption of DBSA on the skin.

TABLE III.—EFFECT OF pH AND CONCENTRATION ON THE ADSORPTION OF HEXACHLOROPHENE AND DBSA

Surgical Scrub[a]	Hexachlorophene, %	DBSA, %	pH	—Mcg. Adsorbed/Sq. Cm. Skin— Hexachlorophene	DBSA
No. 1681	1.0	24	3.0	23	565
No. 1692	1.0	24	5.0	19	400
No. 1691	1.0	24	7.0	10	185
Phisohex[b]	3.0	..	5.5	27	...
No. 1531A	3.0	24	3.0	44	435
No. 1291	3.0	48	3.0	48	850
Septisol[c]	0.75	..	9.4	16	...
Gamophen[d]	2.0	..	9.5	14	...

[a] Experimental surgical scrub base = DBSA, lauric diethanolamide 4%, isopropyl lanolin ester 1%, citrate buffer 2%, alcohol 12%, water.
[b] Phisohex base = sodium octylphenoxyethyl ether sulfonate, lanolin cholesterols, petrolatum, water.
[c] Septisol base = vegetable oil soap, water.
[d] Gamophen base = lanolin 4%, anhydrous soap.

The blank (background) correction for DBSA was large on the Ivory-scrubbed wool, indicating a liberation from the wool of an amino acid, probably tyrosine, absorbing in the ultraviolet region of DBSA. The indication is that the results being obtained with the 30-extraction procedure are of the proper order of magnitude.

Effect of pH and Concentration on Adsorption.—Using a scrub containing 1% hexachlorophene and 24% DBSA, it can be seen from Figs. 2 and 3 that the pH of the scrub product has a pronounced effect on the hexachlorophene and DBSA adsorbed. At low pH, adsorption continues even after prolonged scrub periods, but at pH 7 the rate of adsorption does not increase after eight minutes of scrubbing. For comparative purposes, Table III shows results obtained with several scrubs at a ten-minute cumulative scrub time. These results are shown graphically in Figs. 4–6.

At pH 3, and in the presence of 3% hexachlorophene, the DBSA adsorbed on the skin is directly proportional to the concentration in the scrub product, i. e., doubling the concentration increases the DBSA by a factor of 1.96. Hexachlorophene adsorption increases at a somewhat slower rate; tripling the concentration approximately doubles the adsorption.

Removal of DBSA and Hexachlorophene from the Skin With Soap.—As shown in Figs. 4 and 6, scrubbing with Ivory soap will completely remove DBSA and hexachlorophene from the skin. This removal is complete even after several weeks' exclusive use of these materials.

The pH of the Skin After Scrubbing.—The pH of the skin was determined immediately after these scrubs and subsequently to determine to what extent the skin surface pH was maintained. After a six-minute scrub with the buffered product of pH 3, the pH of the skin surface drops to 5.03, from an initial pH of 6.05. This is evidence of the well-known buffer capacity of the skin surface and demonstrates that merely lowering the skin surface pH by 1 unit will highly cationize the skin surface via salt formation at the nitrogen sites, leading to greater adsorption of both hexachlorophene and DBSA. For the effect of soap, Fig. 7 shows that the skin surface pH after a six-minute Ivory soap scrub is 7.52 (from 6.05) and that this pH drops off rapidly with time compared to the slower rise after the acid scrubs. Since the products of skin respira-

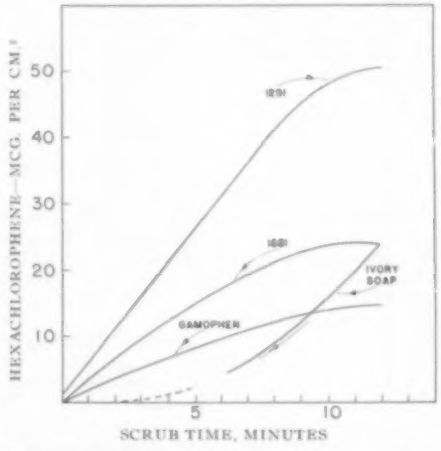

Fig. 4.—Adsorption of hexachlorophene from various surgical scrubs.

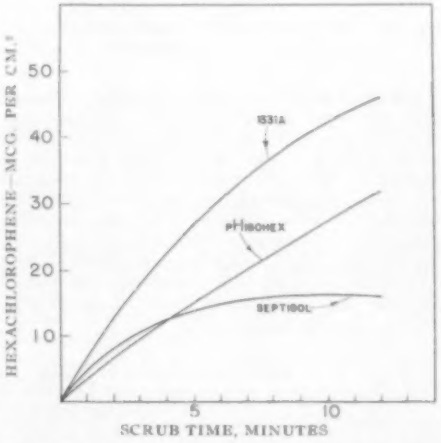

Fig. 5.—Adsorption of hexachlorophene from various surgical scrubs.

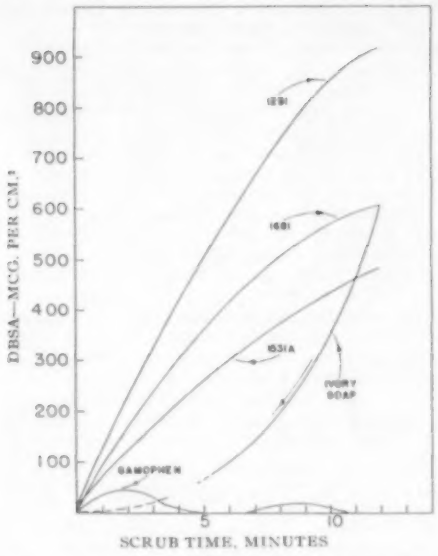

Fig. 6.—Adsorption of DBSA from various surgical scrubs.

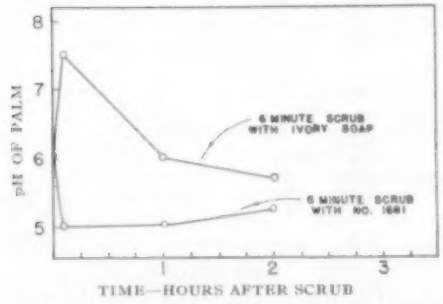

Fig. 7.—The pH of the skin after scrubbing.

tion and metabolism are acidic (carbon dioxide, sweat, sebum) these may serve to hasten the more rapid drop in pH (16) after the soap scrub.

Adsorptive Capacity of Various Skin Sites.—To evaluate any differences in the adsorptive capacity of various skin areas, and between different species, a series of scrubs was run with the results as shown in Table IV.

This comparison is made at the cumulative ten-minute scrub level, using surgical scrub No. 1681. The results over the full scrub period are shown in Figs. 8 and 9. A great difference in adsorption of hexachlorophene and DBSA on various skin sites is indicated, the most notable difference in man being the much greater adsorption on the more highly keratinized areas of palm and finger tips, compared to the forearm. There is greater adsorption of hexachlorophene on the thumb, and less adsorption on the second finger, when the finger

TABLE IV.—THE ABSORPTIVE CAPACITY OF VARIOUS SKIN SITES

Substrate and Test Area	Mcg. Adsorbed/Sq. Cm. Skin Hexachlorophene	DBSA
Man, palm	23	565
Man, finger tips	24	360
Man, forearm	5	95
Albino rabbit, flank (depilated with thioglycolate)	5	78
Albino rabbit, flank (shaved)	4	0
Albino guinea pig, flank (shaved)	4	20

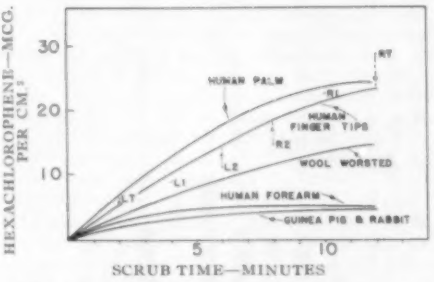

Fig. 8.—Adsorption of hexachlorophene on various substrates. *LT* = left thumb; *L1* = left 1st finger; *L2* = left 2nd finger, etc.

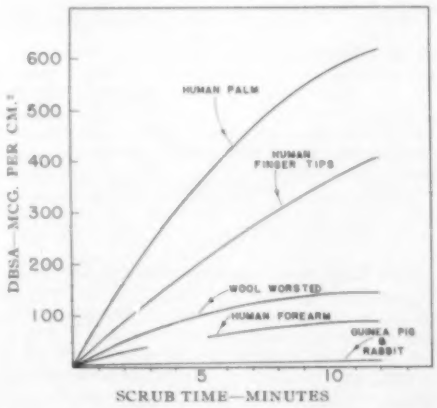

Fig. 9.—Adsorption of DBSA on various substrates.

tips are compared among themselves. The shaved flanks of the rabbit and guinea pig show little adsorption even though some hair is present which cannot be removed without damaging the skin.

Adsorption Levels With Prolonged Use.—Hexachlorophene determinations were run on the skin as exclusive scrubbing with a surgical bar soap[3] containing 2% hexachlorophene continued over a period of several days. Thus, experimental data

[3] Gamophen Surgical Soap, Ethicon, Inc., Somerville, N. J.

were gathered which give a basis for comparing the rate of adsorption of hexachlorophene with its rate of disappearance from the skin. The results are shown graphically in Fig. 10. Table V shows comparable results obtained with a low-pH, DBSA-based scrub.

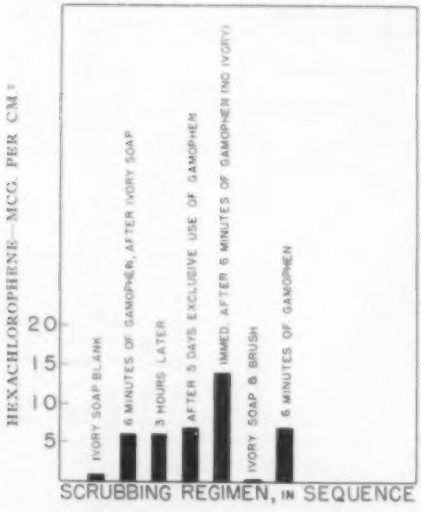

Fig. 10.—The "cumulative" effect of hexachlorophene.

TABLE V.—EXCLUSIVE USE OF HEXACHLOROPHENE SCRUBS

Surgical Scrub	Elapsed Time	Hexachlorophene Absorbed, Mcg./Cm.²
Gamophen soap	6 min. (Initial scrub)	7
Gamophen soap	5 days	7
No. 1681	6 min. (Initial scrub)	16
No. 1681	5 days	15

Ionic Reaction of Hexachlorophene at Low pH.—The experiment below was carried out in order to study the extent to which hexachlorophene undergoes ionic reactions in acid solution, and to obtain data useful for postulating a mechanism helpful in considering the substantivity of hexachlorophene to the skin in relation to the skin substantivity of other compounds.

Equimolar amounts of the particular compound and a high molecular weight quaternary ammonium chloride compound, Hyamine 2389[4] (C_9 – C_{12}-alkyltolylmethyl trimethylammonium chloride) were dispersed in water with the aid of alcohol. The dispersions were diluted out to a concentration of 0.02 *N* and were shaken out once with xylene to remove insoluble material from the reaction mixture. The pH was taken with the glass electrode. The results are shown in Table VI and in Fig. 11.

[4] Rohm & Haas Co., Philadelphia, Pa.

TABLE VI.—REACTION OF ANIONIC COMPOUNDS WITH CATIONIC SURFACTANT

Compound	Molecular Weight	pH Dispersed Without Quaternary	pH Dispersed With Quaternary
Hexachlorophene	407	5.3	2.4
Bithionol	356	6.1	2.2
Oleic acid	282	5.0	3.0
o-Benzyl *p*-chlorophenol	219	6.6	4.9
o-Phenylphenol	170	6.4	5.8
Cresylic acid	108	6.3	5.8

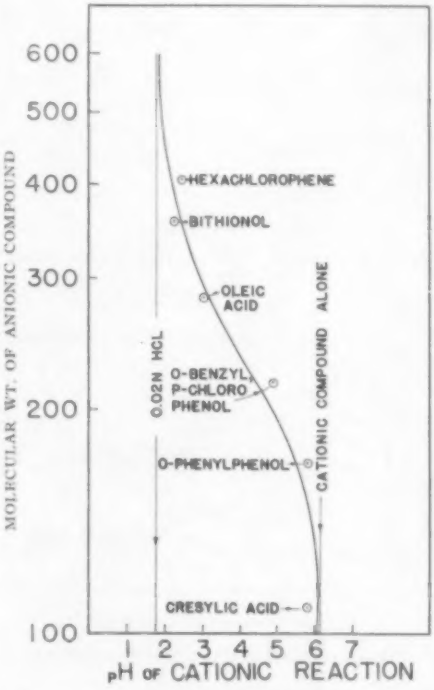

Fig. 11.—Relationship of molecular weight to anion-activity as evidenced by pH drop with cationic compound.

DISCUSSION

Mechanism of the Adsorption of DBSA.—From studies carried out with the proteinaceous fibers, silk, wool, and hair (15, 17–19), and with other proteins such as insulin (20), albumin, gelatin, casein, and zein (21), it appears that anionic surfactants can act (as regards adsorption) upon proteins in two ways: (*a*) at low pH or on the acid side of the isoelectric point of the protein, a straightforward metathetical chemical reaction takes place (chemisorption) between the negatively charged ion of the anionic surfactant molecule and a cationic nitrogen site. Heats of reaction measured are in agreement with this chemisorption reaction; and (*b*)

on the alkaline side of the protein isoelectric point a physical adsorption takes place with weaker forces holding the protein surfactant together. This would be a weak combination of nonpolar groups of protein and hydrophobic residue of surfactant, similar to the forces of micelle formation (21). The results of this study are in agreement with this theory since an increased degree of substantivity of the DBSA to the skin is found as the pH is lowered. The keratin or other skin protein of the hands follows the general adsorption reactions of proteins and has an isoelectric point, as Rothman (22) points out, probably between pH 4 and pH 5. Both adsorption mechanisms also appear to play a role in the action of the DBSA on bacteria. Flett, *et al.* (23, 24), found that kerylbenzenesulfonate lost most of its bactericidal action in alkaline solution and that the greatest bacterial protein-surfactant combination took place at acid pH. Rapid and complete killing occurred below pH 4. Karabinos (25) envisions the acidified bacterium as being "suffocated" as a result of being "coated" by the anionic surfactant.

Mechanism of the Adsorption of Hexachlorophene.—From the structure and ionization constants ($pK_1 = 5.4$; $pK_2 = 10.9$) (26), we might expect the adsorption of hexachlorophene on the skin to be largely of the type discussed in (*b*) above, but somewhat stronger, perhaps, due to a hydrogen-bonding between phenolic hydroxyl and the —NH— groups in the protein backbone (27). The results reported here indicate that a common denominator exists between molecular weight, skin substantivity, antibacterial efficacy, and dependence on pH for hexachlorophene and other compounds of its class.

The interrelation of these properties is best understood by considering the anion-active nature of compounds such as benzoic acid, fatty acids, salicylic acid (25), and higher molecular weight phenols on the one hand, and the fact that the skin surface and bacteria (28, 29) are largely proteinaceous and are cation-active at low pH.

For an effective reaction between skin or bacterial protein the anionic reactant must be high enough in molecular weight to be anion-active. This weight is usually such that solubility is low and micelle formation occurs. Kunz and Gump (30) noted that prior to the invention of hexachlorophene, phenols were not suitable as additives (i. e., in small proportion) to soap for the formulation of antibacterial soaps to be used on the skin. It is likely that these phenols were too low in molecular weight for adequate skin substantivity. By the analogy of a quaternary ammonium compound to the cationized skin or bacterial protein, the results given in Fig. 11 show the effect that molecular weight may have (in a series of active compounds) on skin substantivity and antibacterial action. The hexachlorophene, or other compound having a potentially ionizable hydrogen atom, reacts with the quaternary ammonium chloride to yield a high molecular weight, insoluble compound and hydrochloric acid: $R_4NCl + HO\phi = R_4NO\phi + HCl$. With a low molecular weight hydrogen ion source, such as cresylic acid, the weight of the anion is too low to form an insoluble compound and only a slight amount of HCl is liberated. As the molecular weight of the anionic compound (hydrogen source) increases, the reaction product with the cationic compound becomes more insoluble and the HCl concentration rises. Since a high molecular weight is necessary for skin substantivity, it can be seen that a scrub product could be effective on inanimate objects where substantivity is less important and yet be unsuitable for use on the skin.

It is well established, in testing phenols for antibacterial activity, that lowering the pH (31) increases the efficacy of the phenol. That is, the killing dilution of phenols becomes much greater at low pH where even such acids as HCl and H_2SO_4 are ineffective by themselves. Since this study has shown that the hexachlorophene adsorbed (Fig. 2) is a function of pH, it would indicate that an ionic mechanism was operating here, i. e., bacteria and the skin surface undergo the reactions of proteins and we cannot consider that we acidify a phenol in the presence of bacteria without recognizing that we acidify the bacterial protein, hence cationize it, and bring about a greater reactivity with the phenol anion. It would be more helpful, then, in understanding the bactericidal process as concerns the relationship between pH and phenols (and other anionic agents) to speak of "cationizing the bacteria" rather than "acidifying the phenol" or other active anion.

In like manner, lowering the pH of the skin increases the cationicity of the skin protein and causes greater reactivity with the hexachlorophene anion. In a dispersed system, even at pH 5 and pH 3, then (in the undissociated form) we can consider hexachlorophene to be an anion-active compound. Also, Pankhurst (27), considering the tanning action of phenols on hides (i. e., collagen) finds there is evidence of ionic interaction in vegetable (phenol) tanning.

Cumulative Action of Hexachlorophene.—Workers in the field of degerming (10) the skin frequently speak of a "cumulative action" (32, 33) of hexachlorophene on the skin, by which it is meant, presumably, that the hexachlorophene cumulates on the skin (with exclusive use of a hexachlorophene soap) and builds up or concentrates toward ever-higher levels until a low plateau is reached on the bacterial count-time curve. The actual skin adsorption levels obtained while scrubbing exclusively with a hexachlorophene soap (Fig. 10) may be explained as follows: starting with Ivory soap-clean skin (palms) a six-minute continuous scrub with the surgical bar soap lays down 7 mcg. of hexachlorophene/sq. cm. Three hours later, the hexachlorophene is still about the same level. Five days later, after exclusive use of the soap, the hexachlorophene level is only slightly higher (mid-morning). When this mid-morning skin is scrubbed for six minutes, the adsorption doubles, from 7 to 14 mcg./sq. cm. skin. Vigorous scrubbing with Ivory soap then brings the hexachlorophene down to zero. Again, six minutes with surgical bar soap brings the hexachlorophene back to 7 mcg./sq. cm. skin.

If a cumulation of hexachlorophene were taking place on the skin, then by definition, a significantly higher amount of hexachlorophene per unit area of skin should be present after a given period of time. The results of the present work show that: (*a*) after five days of exclusive use of the surgical bar

soap there is no increase in concentration of hexachlorophene on the skin; the rate of use of the surgical soap is counterbalanced by the rate of disappearance; (*b*) a higher level of hexachlorophene is attained when the soap is used on a normal (acid) skin than on an alkaline (Ivory-scrubbed) skin. It would appear that the concentration of hexachlorophene on the skin is not so much a function of the number of days elapsed since the first scrubbing was done as it is on the condition of the skin, length of scrubbing time, and the time-lapse since the previous scrub. The largest use of hexachlorophene is in soaps intended to be used over an extended period of time, and practically all of the bacteriological work done which considers the "cumulative" effect of hexachlorophene is on soap-based products. Although low-pH, DBSA-based surgical scrubs lay down on the skin far more hexachlorophene than a soap-based product in a given period of scrubbing as indicated in Table I, it is interesting to note that determinations of hexachlorophene on the palms of four subjects using surgical scrub No. 1681 exclusively for a period of twenty-one days show that there is no cumulation of hexachlorophene at all on the hands over this extended period of time; there is no more hexachlorophene on the skin on the twenty-first day than there was on the first day. Thus, there is no cumulation of hexachlorophene on the skin from either an alkaline soap or an acid syndet.

It follows, then, that the antibacterial effectiveness of hexachlorophene on the skin is not due to a cumulative building-up of this compound on the skin, but rather to its being maintained at a given level on the skin for a long period of time. According to Cade (11) the efficacy of hexachlorophene on the skin lies in its being continuously available in sufficient concentration to lessen the degree of multiplication of the bacteria. It is theoretically possible that the concentration of hexachlorophene on the skin resulting from a single scrubbing with a hexachlorophene soap might be sufficient to obtain a low bacterial count after a lapse of several days if we did not have to be concerned with this concentration being lowered through being rubbed off on clothing, being inactivated (34) or diluted by perspiration or other body exudations, etc. Continuous use of hexachlorophene soap may serve more the purpose of providing a modest concentration of "fresh" hexachlorophene to a site where the hexachlorophene applied earlier had become inactivated or "spent" than the purpose of "building-up" the hexachlorophene concentration to a high level.

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Distribution Coefficients and Dissociation Constants of a Series of Barbituric Acid Derivatives*

By J. P. LEYDA, D. J. LAMB, and L. E. HARRIS

An equation was derived to illustrate the relationship between distribution coefficients and dissociation constants of weak acids. The method used in determining the distribution coefficients of a series of barbituric acid derivatives is described. These data obtained from the distribution studies are used to calculate the thermodynamic dissociation constants of a series of barbituric acid derivatives. The relationship and methods employed are shown to be valid and the results reproducible.

THIS INVESTIGATION was undertaken as the preliminary phase of a study for determining a possible relationship between free energy differences and biological activity. It was the purpose of this investigation to study the relationship between the distribution coefficients and dissociation constants of some barbituric acid derivatives.

Distribution studies have been used to determine dissociation constants of various organic acids and bases (1, 2). Golumbic (1) has derived an equation to illustrate the relationship between distribution coefficients and dissociation constants in a system when the hydrogen ion concentration is insignificant in comparison to the dissociation constant. This equation is not applicable to systems in which the hydrogen ion concentration and dissociation constant are of the same magnitude.

The dissociation constants of barbituric acid derivatives have been determined by Krahl (3), using pH measurements at various ionic strengths, and by Biggs (4), using spectrophotometric measurements at various hydrogen ion concentrations.

THEORY

During this investigation it was necessary to derive an equation to illustrate the relationship between the distribution coefficient and the dissociation constant of a weak acid. An assumption is made and the terms of the system defined. It is assumed that the weak acid is monomeric in the aqueous and organic phases and only the undissociated species distributes between the immiscible solvents.

In this equation, k is the observed ratio of the concentration of weak acid in the aqueous phase to its concentration in the organic phase at a specific pH, k_o is a similar observed ratio at a pH where only the undissociated acid is present, and pKa is the logarithm of the reciprocal of the dissociation constant of the acid.

The following equation can be derived from the relationships between k, k_o, and the Henderson-Hasselbalch equation.

$$\mathrm{pKa} = \mathrm{pH} - \log (k - k_o)/k_o$$

From this equation the apparent dissociation constant of a weak acid can be calculated from the distribution coefficient of the acid at a pH where only undissociated molecules are present and at a pH where partial dissociation of the acid is possible. See Table I.

The true thermodynamic dissociation constant which is based on the activity of the acid may be calculated when the acid concentration is converted into terms of activity. The correction factor used in changing to activity is the mean activity coefficient ($-\log f \pm$).

$$\mathrm{pKa}' = \mathrm{pKa} - \log f \pm$$

This correction factor is the same as used by Biggs (4). The mean activity coefficient can be calculated by the Davies equation (5).

EXPERIMENTAL

Materials.—The barbiturates were recrystallized from ethanol-water mixtures with the exception of butethal. Due to the low melting point of this compound a mixture of ether-petroleum ether (30–60° b. p.) was employed. Tris (hydroxymethyl) amino methane, commonly referred to as "tris," was purified by the method described by Fossum (6).

The chloroform used in these studies was purified by washing with a sodium bicarbonate solution, distilled water, and a saturated solution of sodium chloride, respectively. It was partially dried with the addition of anhydrous magnesium sulfate and then distilled. The isooctane used was of reagent grade and the water employed was demineralized and double distilled.

Spectrophotometric Assay.—In solutions of a pH of 9 or below barbiturates have extremely low extinction coefficients. With the addition of an alkali to the aqueous barbiturate solutions to give a pH of about 11 the absorption increases due to the enol formation (7).

A known concentration of barbiturate was dissolved in an alkaline buffer with the pH of 10.9. The wavelength of maximum absorption

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This paper was the winner of the Lunsford Richardson Pharmacy Award (Eastern Division).
Presented to the Scientific Section, A. PH. A., Cincinnati meeting, August 1959.

TABLE I.—DATA PERTAINING TO A SERIES OF BARBITURIC ACID DERIVATIVES USED IN THE DERIVED EQUATION

Generic Name	R	R′	Max., mμ	Molar Extinction, E × 10^{-3}	k_o	k	pKa Found	pKa Lit.
Barbital	Et	Et	239	10.2	9.06	21.62	8.06	7.97 (4)
Probarbital	Et	isopropyl	240	9.8	2.49	5.12	8.17	8.01 (3)
Butethal	Et	*n*-butyl	239	11.5	0.47	0.99	8.10	7.92 (3), 7.98 (4)
Butabarbital	Et	*sec*-butyl	240	11.0	0.46	0.96	8.16	....
5-Ethyl-5-(2-methyl)-allylbarbituric acid	Et	2-methyl allyl	240	9.6	1.60	5.29	7.85	....
Pentobarbital	Et	1-methyl butyl	240	10.1	0.17	0.35	8.17	8.11 (3)
Vinbarbital	Et	1-methyl butenyl	239	9.5	0.64	2.58	7.72	....
Amobarbital	Et	isoamyl	239	10.0	0.16	0.40	8.02	7.96 (4), 7.94 (3)
Cyclobarbital	Et	cyclo-1-enyl	239–240	10.6	0.61	2.10	7.79	7.50 (3)
Phenobarbital	Et	phenyl	239	11.0	1.58	9.14	7.54	7.45 (3)
Diallylbarbituric acid	Allyl	allyl	241	9.2	2.30	7.25	7.88	7.77 (3)
Aprobarbital	Allyl	isopropyl	241	9.2	1.32	3.09	8.09	7.99 (3)
Allylbarbituric acid	Allyl	isobutyl	240	9.5	0.41	1.35	7.86	....

and the molar extinction coefficients were determined by measuring the absorbance of known concentrations of barbiturates at 239, 240, and 241 mμ. The measurements were obtained by the use of the Beckman DU spectrophotometer.

These data were used in assaying the aqueous phases during the distribution studies.

Buffer Solutions.—The preparation of the tris and acidic buffer solutions of a definite ionic strength followed a modification of the general scheme shown by Bates (8). The alkaline buffer solutions were prepared with the weak base tris, hydrochloric acid, and either potassium or sodium chloride added to give the desired ionic strength. The acidic buffers were prepared using the hydrochloric acid and sodium or potassium chloride to adjust the ionic strength. The ionic strength of the buffer solutions were calculated in the usual manner.

The ultimate standard employed in determining pH was a phosphate buffer described by Bates (9). The pH of the tris buffer solutions were determined at 25° with a Beckman GS pH meter.

Distribution Studies.—The solvent system used was an aqueous buffer phase and a mixture of chloroform (60%) and isooctane (40%). To determine the distribution coefficient (k_o) of the undissociated acid the acidic buffer was used as the aqueous phase. The tris buffer at a known pH was used as the aqueous phase in determining the distribution coefficient (k).

A stock solution of barbiturate (approx. 9×10^{-4} but known) was prepared in the buffer solutions. Equal portions of the organic phase and the stock barbiturate solution were placed in 10-dram Opticlear vials. The vials were sealed and placed in a constant temperature bath at 25 ± 0.01° to equilibrate. When temperature equilibrium was reached the vials were shaken for one minute. The phases were allowed to separate completely and then an aliquot of the aqueous phase was removed, diluted with pH 10.9 buffer to give a suitable absorbance, and measured spectrophotometrically. The original stock solution was assayed in a similar manner.

The following equation was used to calculate the k and k_o

$$k \text{ or } k_o = \frac{A \text{ aqueous phase after distribution}}{A \text{ stock solution} - A \text{ aqueous phase}}$$

where A represents the absorbance. Direct substitution of the measured pH, k, and k_o into the derived equation gave the apparent pKa′ of the barbiturate. Upon correction to activities by the Davies equation the true thermodynamic pKa was obtained. The ionic strength of the aqueous buffer solutions was 0.02.

DISCUSSION AND RESULTS

The equation which was derived to correlate the relationship between the distribution coefficients and the dissociation constants of weak acids was based on the assumption that the acid was present in the monomeric form in the aqueous and organic phases and only the undissociated species distributes between the immiscible solvents. This assumption was shown to be valid due to the agreement between the literature dissociation constants and the experimentally determined values.

This equation is more applicable than previously derived relationships since the hydrogen ion concentration can be of the same or different magnitude than that of the dissociation constant. The pH of the aqueous phase has no confined limits as long as the acid is monoprotic. In systems which contain a nonmonoprotic acid the pH must be maintained at a value where it can be assumed that the effect of other ionizable groups is insignificant.

SUMMARY

1. An equation was derived to correlate the relationship between distribution coefficients and dissociation constants of weak acids.

2. The wavelength of maximum absorbance and the molar extinction coefficients of thirteen barbiturates were determined.

3. The dissociation constants of a series of barbituric acid derivatives were calculated. The distribution coefficients of the barbiturates were determined at 25° in a system of aqueous solution, and a mixture of 60 per cent chloroform and 40 per cent isooctane.

4. The assumption on which the relationship between distribution coefficients and dissociation constants of weak acids was based was shown to be true.

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Correlation of the Distribution Coefficients of Various Barbituric Acids*

By DONALD J. LAMB and LOYD E. HARRIS

Evidence is provided to indicate that the distribution coefficients of various barbituric acids can be related to the number of carbon atoms and the Taft polar constants of the 5,5-substituents. This suggests that the hydrophilic portion of the molecule is affected not only by the size of the substituents but, also, by their polarity.

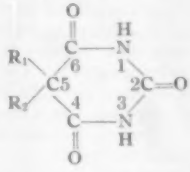
ATTEMPTS have been made to develop relationships between changes in structure and the distribution of compounds between an organic solvent and water. Collander (1) tabulated numerical estimates of the effects of various groups on the distribution coefficients of organic compounds. Alders (2) showed the logarithm of the distribution coefficient to be a linear function of the number of carbon atoms in the alkyl groups of a series of homologous straight-chain organic compounds. However, he reported that deviations from linearity do occur when the alkyl chain contains less than three or four carbon atoms. This, he postulated, is due to differences in inductive effects in lower members of the alkyl side-chain and this difference rapidly decreases as the number of carbon atoms increases.

The purpose of this paper is to develop a relationship of the distribution coefficients of a series of barbituric acids as a function of inductive or polar effects as well as the number of carbon atoms in the 5,5-substituents of these compounds.

DISCUSSION

If a series of compounds, such as 5,5-substituted barbituric acids, is chosen which differ from one another only in carbon content of their substituents, it would be expected that the distribution of these compounds would change with the alteration of the lipophilic character of the substituents as well as the influence these groups exert on the rest of the molecule.

This influence exerted by the substituents on the rest of the molecule may be in the nature of resonance, steric, or inductive (polar) effects. In the 5,5-substituted barbituric acids used in this study the 5-position of the barbituric acid ring is saturated and, therefore, would be incapable of transmitting any resonance effects from the substituents to the carbonyl groups in the ring.



Here the carbonyl groups, in positions 4 and 6, are defined as the "reaction sites." Since the 5,5-substituents are not adjacent to these reaction sites, it would seem that steric inhibition of solvation of the hydrophilic portion of the molecule would be

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TABLE I.—CORRELATION OF THE EFFECT OF STRUCTURE ON THE DISTRIBUTION COEFFICIENTS OF 5,5-SUBSTITUTED BARBITURIC ACIDS BY EQ. 1

	Compound	R_1	R_2	N	$\Sigma\sigma^*$	Log K Observed	Log K Calcd.
1.	Barbital	C_2H_5	C_2H_5	4	−0.225	+0.957	+0.958
2.	Probarbital	C_2H_5	i-C_3H_7	5	−0.240	+0.396	+0.331
3.	Butethal	C_2H_5	n-C_4H_9	6	−0.245	−0.328	−0.242
4.	Butabarbital	C_2H_5	$C_2H_5(CH_3)CH$	6	−0.255	−0.337	−0.296
5.	Pentobarbital	C_2H_5	$C_3H_7(CH_3)CH$	7	−0.255	−0.770	−0.842
6.	Amobarbital	C_2H_5	i-C_5H_{11}	7	−0.245	−0.796	−0.788
7.	Phenobarbital	C_2H_5	C_6H_5	8	+0.04	+0.199	+0.199
8.	Aprobarbital	CH_2=$CHCH_2$	i-C_3H_7	6	−0.185	+0.121	+0.082
9.	Allylbarbituric acid	CH_2=$CHCH_2$	i-C_4H_9	7	−0.190	−0.387	−0.491
10.	Diallylbarbituric	CH_2=$CHCH_2$	CH_2=$CHCH_2$	6	−0.120	+0.362	+0.431
11.	5-Ethyl-5-(2-methyl)-allylbarbituric acid	C_2H_5	CH_2=$C(CH_3)CH_2$	6	−0.175	+0.204	+0.135

slight or at least constant for the series studied. Inductive or polar effects then would be the main effects exerted by the substituents.

If these assumptions are considered valid, the following equation may be written

$$\text{Log } K = \gamma N + \rho^* \Sigma \sigma^* + \log K_o \quad \text{(Eq. 1)}$$

where K is the distribution coefficient of the barbituric acid between aqueous acid solution and 40% isooctane-chloroform solution. These data and methods for their determination are reported elsewhere (3). Log K is the distribution coefficient of the unsubstituted barbituric acid, N is the number of carbon atoms in the 5,5-substituents, and $\Sigma\sigma^*$ is the sum of the Taft polar constants (4). Gamma and ρ^* are "reaction constants" indicating the susceptibility of the "reaction" or distribution coefficient to a change in the number of carbon atoms and polarity, respectively.

Equation 1 is analogous to that reported by Taft (6) to describe the rates of methanolysis of a series of l-methyl esters in terms of the polar and steric effects of the acyl component of the esters.

The polar constants for the series of eleven 5,5-substituted barbituric acids listed in Table I were obtained from published data (6). They were chosen considering the 5,5-substituents and the carbon atom in position five of the ring as a unit, i. e., the polar constant for aprobarbital (5-allyl-5-isopropylbarbituric acid) was obtained from the sum of the polar constants for the CH_2=$CHCH_2$-CH_2— and $(CH_3)_2CH_2CH$— groups rather than the allyl and isopropyl groups. This is justified in that the value listed for the $(C_2H_5)CH$— group is −0.225 while doubling the value of the n-propyl group leads to −0.230. Since the polar constant for a 3-butenyl group was not available, its value was calculated from the pKa of vinyl acetic acid (7) and the equation describing the ionization constants of carboxylic acids as a function of the polar constants of their substituents (5). The value obtained was +0.17; the value of the CH_2CH—CH— group is +0.36, and that for the CH_2CH—$CHCH_2$— group is +0.13, therefore, introduction of a methylene group decreases the constant by 0.23. Combining this with the value of +0.17 for the allyl group leads to a value of −0.06 for the CH_2=$CHCH_2$-CH_2— group. By similar methods a value of −0.055 was obtained using the pKa of allyl malonic acid (7) after first subtracting the constant of the $HOOCCH_2$— group.

The number of carbon atoms of the 5,5-substituents were used without addition of the carbon in position five of the ring as a matter of expedience since addition of a constant number to the value of N would have no overall effect.

The constants of Eq. 1 were determined by standard statistical procedures as conveniently outlined by Taft (6). The values obtained along with their standard deviations are $\gamma = -0.5462 \pm 0.0006$, $\rho^* = 5.38 \pm 0.09$, and $\log K_o = 4.353 \pm 0.036$. The multiple correlation coefficient is 0.997 and the probable error of the fit of a single observation is 0.08 log unit. The values of log K calculated by this equation are listed in Table I together with the experimentally determined values.

It is evident that the distribution coefficients of the 5,5-substituted barbituric acids are affected by more than the change in carbon number of their substituents (see Fig. 1). When a correction is applied for the polar effects of the substituents, a linear relationship is obtained (Fig. 2). Considering this linear relationship and the magnitude of the

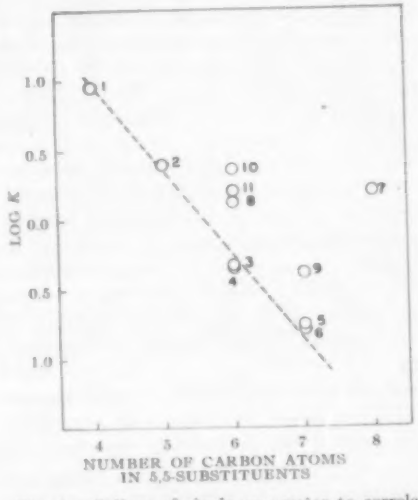

Fig. 1.—Failure of single parameter to correlate the distribution coefficients of 5,5-substituted barbituric acids. The numbers adjacent to the circles correspond to the compounds listed in Table I.

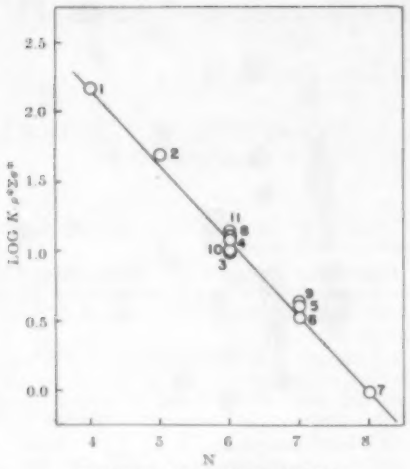

Fig. 2.—Correlation of the distribution coefficients of 5,5-substituted barbituric acids by the equation $\log K - \rho^* \Sigma\sigma^* = \gamma N - \log K_0$. The numbers adjacent to the circles correspond to the compounds listed in Table I.

polar reaction constant ($\rho^* = +5.38$), small changes in the inductive effects of the lipophilic portion of the molecule can have a pronounced effect on the reaction site or hydrophilic portion of the molecule. The excellent correlation of these two parameters with the experimental data would indicate that steric effects, as expected, are constant or negligible in this reaction series.

Equation 1 emphasizes the fact that the carbon atom number and the polar effects are independent of each other, i. e., if one is constant or zero, the other defines the changes in log K. This is illustrated in Fig. 2 with compounds 3, 4, 8, 10, and 11 all having a value of $N = 6$ and the polar constant changing by a factor of more than 2. The fit of the data to the theoretical line is in good agreement as predicted by the equation. On the other hand, when the polar constants are nearly constant as in compounds 1, 2, 3, 4, 5, and 6, a linear relationship is obtained (Fig. 1) from the plot of Log K *versus* number of carbon atoms.

Equation 1 can be used with some confidence in predicting the distribution coefficients of barbituric acid derivatives substituted in position five by hydrocarbon groups provided the polar constants are available or a reasonable estimate can be made.

SUMMARY

An equation has been derived to establish the distribution coefficients of a series of 5,5-substituted barbituric acids as functions of the number of carbon atoms and Taft polar constants of their substituents. The correlation coefficient indicates an excellent fit of the data to the equation with a probable error of a single observation of 0.08 log unit. This indicates that the distribution of the barbiturates is affected not only by the mass of the 5,5-substituents but, also, by the influence of the inductive effects of these groups on the hydrophilic portion of the molecule. It has also been shown that the two parameters which define the distribution coefficients are independent of each other as predicted by Eq. 1.

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An Evaluation of Certain Hypotensive Agents IV*

Diquaternarized Piperidine Derivatives

By J. P. BUCKLEY, M. L. JACQUART, R. K. BICKERTON†, W. J. HUDAK‡, F. M. SCHALIT†, J. J. DEFEO§, and J. A. BIANCULLI

Certain diquaternarized piperidine derivatives and several bis-tertiary amines were evaluated in the anesthetized rat and dog. The most active hypotensive agents were: 3-morpholinopropyl N-methylpipecolinate dimethiodide (JB-643), 2-morpholinoethyl N-methylpipecolinate dimethobromide (JB-654), and beta(3-diethylamino-1-propynyl)-N-methylpiperidine dimethobromide (JB-579). The compounds JB-643 and JB-654, administered orally to unanesthetized dogs, produced marked hypotensive responses persisting for more than eleven hours.

CERTAIN diquaternarized piperidinecarboxylic acid esters have previously been reported by our laboratory to be potent hypotensive agents (1–3). Beta-dimethylaminoethyl N-methylpipecolinate (JB-591) has also been reported to possess both central and peripheral hypotensive properties and to be effective in controlling essential hypertension in human patients (2). This present report deals principally with the evaluation of additional diquaternarized piperidinecarboxylic acid esters, certain of their "reversed" esters, and some β-dialkylaminoalkynyl-N-methylpiperidines (Fig. 1).

EXPERIMENTAL

Hypotensive Activity in Rats.—The experimental compounds were screened and evaluated in anesthetized normotensive rats using the method described in a previous paper (4).

The oral activity of JB-654 was also investigated. The compound was administered via gastric intubation to rats prepared as follows:

(*a*) Rats were anesthetized with urethan, 1.2 Gm./Kg., intraperitoneally and the carotid blood pressures were recorded on a Sanborn Viso-Cardiette utilizing a Sanborn electromanometer.

(*b*) Blood pressures of unanesthetized rats were determined via a photoelectric tensometer (Metro Industries) or by direct recording utilizing the method of Still, *et al.* (5). Direct blood pressures were obtained via a small length of polyethylene tubing (PE-10, Clay Adams) permanently inserted into the abdominal aorta.

Hypotensive Activity in Dogs.—Several of the compounds were further evaluated in normotensive dogs to study the possibility of species variation.

(*a*) Mongrel dogs were anesthetized with pentobarbital sodium, 35 mg./Kg., i. v., and the blood pressures were recorded via a femoral artery. Fresh solutions of the compounds were administered via a femoral vein.

(*b*) The oral activity of JB-643 and JB-654 was investigated in unanesthetized normotensive dogs. Mongrel dogs were anesthetized with 150 mg./Kg. of hexobarbital sodium, i. v. An incision approximately 1 cm. in length was made through the skin just above and perpendicular to the femoral artery. A segment of the artery, approximately 4 cm. long, was carefully isolated and a sterile nasal oxygen catheter (Bardic 10) inserted into the lumen to an approximate depth so that it entered the abdominal aorta and was affixed by a double ligature. The catheter had previously been cut to the proper length, filled with sterile heparin solution (1,000 U. S. P. units per ml.), and the plastic adapter and cap, or B-D L/609 adapter and 417A male plug, inserted. The catheter was sutured to the skin of the leg, the incision closed, and the entire leg wrapped with sterile gauze and then with an elastic bandage. Procaine penicillin G, 100,000 units, was administered intramuscularly into the opposite leg and the dog was permitted to recover for a minimum period of two days before being utilized. The dogs were immobilized by suspending them in a canvas sling from a suitable height. Direct blood pressures were obtained by connecting a sterile saline system to the nasal catheter and were recorded via a Sanborn electromanometer or a mercury U-tube manometer. The compounds were administered in capsule form to dogs fasted for twenty-four hours.

RESULTS AND DISCUSSION

Hypotensive Activity in Rats.—The hypotensive activity of the compounds, administered intravenously, is summarized in Table I. The most potent compounds investigated in this present series were JB-643 and JB-654 (Fig. 2), two diquaternarized esters of the piperidinecarboxylic acid. This agrees with the results obtained in our laboratory with a similar series of compounds (1), in that the diquaternarized esters of piperidinecarboxylic acid, having the side chain containing two or three methylene units (CH_2) attached to a quaternarized dimethylamino or morpholino group linked in the 2-position of the piperidine moiety, were the most

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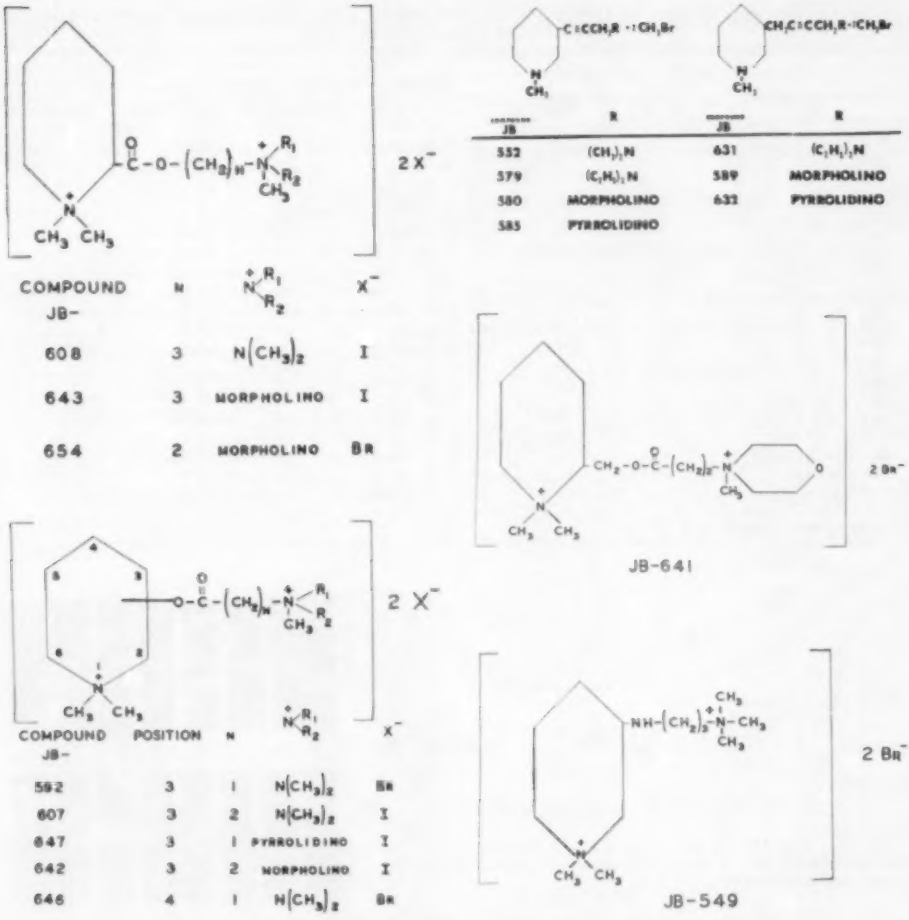
2 X⁻
COMPOUND JB- | N | X⁻
608 | 3 | N(CH3)2 | I
643 | 3 | MORPHOLINO | I
654 | 2 | MORPHOLINO | BR
JB | R | JB | R
532 | (CH3)2N | 631 | (C2H5)2N
579 | (C2H5)2N | 589 | MORPHOLINO
580 | MORPHOLINO | 632 | PYRROLIDINO
585 | PYRROLIDINO
2 Br⁻
JB-641
2 X⁻
COMPOUND JB- | POSITION | N | X⁻
592 | 3 | 1 | N(CH3)2 | BR
607 | 3 | 2 | N(CH3)2 | I
647 | 3 | 1 | PYRROLIDINO | I
642 | 3 | 2 | MORPHOLINO | I
646 | 4 | 1 | N(CH3)2 | BR
2 Br⁻
JB-549

Fig. 1.—Structures of the compounds investigated.

active compounds. It appears that the replacement of the terminal dimethylamino group (JB-591) with a morpholino radical (JB-654) not only maintained but also increased the hypotensive activity. The "reversed" esters of N-methylpiperidinecarboxylic acid derivatives, which were investigated, had the side chain linkage at the 3 or 4 position. The most potent compounds were the morpholinoethyl (JB-642) and pyrrolidinomethyl (JB-647) derivatives linked at the 3 position. These "reversed" esters were generally not as potent as the diquaternarized esters of piperidinecarboxylic acid previously reported (1). JB-579, β - (3 - diethylamino - 1 - propynyl) - N - methylpiperidine was the most active compound of a series of β-dialkylaminoalkynyl-N-methylpiperidines and was approximately six times as potent as the diethylamino-2-butynyl derivative (JB-631). The following compounds, not listed in Table I, were found to be relatively inactive: N'-methyl-3-piperidylmethyl N-methylnipecotate dihydrochloride (JB-559), N-methyl-3-piperidylmethyl N-methylnipecotate dimethobromide (JB-609), N-methyl - 3 - piperidyldimethylaminopropionate dimethobromide (JB-593), N-methyl-3-piperidyl dimethylaminoacetate dimethobromide (JB-606), 3-dimethylaminopropyl N-methylnipecotate bimaleate (JB-648), and 1,5-bis-(diethylamino)-3 pentyl 1'-methylnipecotate trimethobromide (JB-652).

The administration of JB-654, 50 mg./Kg. (10 times the i. v. dose) by gastric intubation to anesthetized normotensive rats, produced a mean drop in blood pressure of 54% with a mean time to return to predrug level of one hundred and eighty minutes. Both the per cent drop and duration of action were slightly greater than that produced by 5 mg./Kg., i. v. JB-654, 50 mg./Kg., administered via gastric intubation to unanesthetized rats produced a mean drop in blood pressure of 54% with a mean time to return to predrug levels of one hundred and seventy-two minutes. The maximum drop in blood pressure occurred between

TABLE I.—THE EVALUATION OF CERTAIN DIQUATERNARIZED PIPERIDINE DERIVATIVES ON THE BLOOD PRESSURE OF NORMOTENSIVE RATS[a]

Compound JB-	Dose, mg./Kg.	No. of Rats	Drop in Blood Pressure, % ± S. D.	Mean Time to Return to Original Levels, Min. ± S. D.	Rating[b]
608	2.0	6	47.7 ± 12.2	69.3 ± 18.0	++
643	5.0	4	51.9 ± 11.9	152.3 ± 52.2	++++
654	5.0	6	42.3 ± 7.5	156.0 ± 51.2	++++
592	25.0	4	19.0 ± 16.9	5.4 ± 2.6	+
607	10.0	4	48.8 ± 2.2	26.9 ± 7.5	+
647	5.0	8	41.9 ± 12.6	58.0 ± 19.1	++
642	5.0	6	44.1 ± 9.9	50.7 ± 17.5	++
646	10.0	4	32.4 ± 9.7	2.0 ± 0.2	+
641	20.0	4	46.1 ± 0.8	87.5 ± 19.2	+++
549	5.0	4	54.4 ± 10.7	108.0 ± 25.3	+++
552	20.0	4	51.5 ± 20.1	74.5 ± 17.9	++
579	10.0	4	46.9 ± 3.8	150.0 ± 48.5	++++
580	10.0	4	48.0 ± 6.7	38.8 ± 15.4	+
585	10.0	4	46.0 ± 17.0	18.8 ± 7.9	+
589	10.0	4	51.0 ± 1.7	42.5 ± 3.3	++
631	10.0	5	46.5 ± 9.2	25.0 ± 8.7	+
632	10.0	6	45.9 ± 8.2	18.7 ± 24.0	+
Hexamethonium	5.0	8	46.5 ± 17.9	30.0 ± 15.2	+
Pentolinium	1.0	11	48.7 ± 18.6	118.5 ± 40.3	+++
Chlorisondamine	0.5	14	44.6 ± 13.8	141.2 ± 51.6	++++

[a] Administered intravenously.
[b] Rating: +, 0–40 minutes' duration; ++, 41–80 minutes; +++, 81–120 minutes; ++++, 121+ minutes.

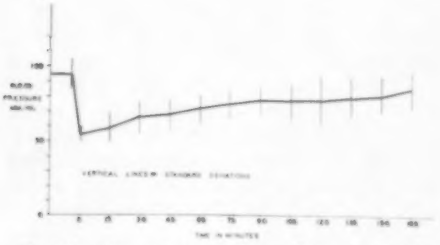

Fig. 2.—The effects of 5 mg./Kg. of JB-654, administered i. v., on the blood pressure of five normotensive albino rats.

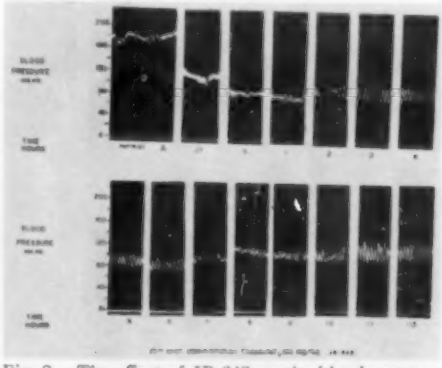

Fig. 3.—The effect of JB-643 on the blood pressure of a normotensive unanesthetized dog.

TABLE II.—THE EFFECTS OF CERTAIN HYPOTENSIVE AGENTS ON THE BLOOD PRESSURE OF NORMOTENSIVE DOGS[a]

Compound JB-	Dose, mg./Kg.	No. of Dogs	Mean Drop in Blood Pressure, %	Time to Return to Original Levels, Min.
608	1.0	3	32	232+
608	2.0	1	58	510+
643	2.5	1	41	246
643	5.0	1	27	174
654	2.5	1	36	142
654	5.0	2	39	350
654	10.0	1	38	352
647	2.5	1	34	165
647	5.0	2	41	185
549	2.5	2	57	180
549	5.0	2	59	750+
552	25.0	4	64	398
579	10.0	1	56	450
580	10.0	1	30	120
589	1.0	1	53	25
589	10.0	1	45	143

[a] Administered intravenously.

thirty and sixty minutes after the compound was administered.

Hypotensive Effects in Dogs.—The hypotensive activity of certain diquaternarized piperidine derivatives administered intravenously is summarized in Table II. The most active compounds were JB-549, JB-608, JB-579, JB-552, JB-654, and JB-643. The results observed in dogs were similar to those observed in rats in that five of the six most active compounds were either 3+ or 4+ in the rats evaluation studies. The only compound which demonstrated a significant difference in activity in the two species was JB-608 which produced marked hypotensive effects in four dogs but only elicited mild hypotensive responses in rats. Since JB-608 is the propyl analog of JB-591, a compound previously reported by Hudak, *et al.*, to possess excellent hypotensive activity (1), it was anticipated that this compound would also be a potent hypo-

tensive drug and it is most probable that the results obtained in the dog may be more significant than the effects of the compound in the rat.

JB-643 and JB-654 produced marked hypotensive effects in unanesthetized dogs when administered via the oral route. JB-643 (100 mg./Kg.) produced drops in blood pressure ranging between 34 and 55% with a mean duration of action in excess of eleven hours (Fig. 3). JB-654 (100 mg./Kg.) produced depressor responses between 31 and 34% with a duration of action in excess of nineteen hours. Both compounds dilated the pupils and relaxed the nictitating membrane. The effects of JB-643 on the nictitating membrane were still evident sixty hours after the administration of the compound. The maximum hypotenive effects produced by both compounds occurred within forty minutes. The results of the oral studies with JB-643 and JB-654 indicates that slightly more than 10% of the compound is initially absorbed.

SUMMARY

1. Twenty-two diquaternarized piperidine and related compounds were evaluated for their hypotensive activity.

2. 3-Morpholinopropyl N-methylpipecolinate dimethiodide (JB-643), 2-morpholinoethyl N-methylpipecolinate dimethobromide (JB-654), β - (3 - diethylamino - 1 - propynyl) - N - methylpiperidine dimethobromide (JB-579) were the most potent hypotensive compounds in this current series.

3. JB-643 and JB-654 exhibited marked hypotensive effects when administered via the oral route to unanesthetized dogs.

4. A method has been described by which direct, continuous blood pressure recordings can be obtained from unanesthetized dogs.

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Studies on *Vinca major* L. (*Apocynaceae*) I*

Isolation of Perivincine

By N. R. FARNSWORTH, F. J. DRAUS†, R. W. SAGER, and J. A. BIANCULLI

Perivincine has been isolated from the leaves and stems of *Vinca major* L. (*Apocynaceae*). The isolation and identification procedures are described, with the identity of perivincine being established by a mixed melting point with a known sample from *Vinca minor*. The optical rotation and pK determinations are reported for this alkaloid, and paper chromatograms were run with the R_f values determined. Preliminary screening of perivincine for hypotensive activity in anesthetized rats indicates that the alkaloid produces a rapid transient drop in carotid blood pressure.

PLANTS OF THE GENUS *Vinca* have been investigated extensively during the past few years with most of the studies being carried out in the search for new alkaloids with potential medicinal uses. Recently several investigators have reported that *Vinca* species possess possible activity in the treatment of hypertension (1), leukemia (2, 3), and cancer (3). We have been interested in the alkaloids of *Vinca major* as a potential source of new medicinal agents. Seven alkaloids have been reported from *Vinca major*. They are reserpine (4), vincamajoridine (5), vincamajoreine (6), vincamajine (7), pubescine, vinine, and another crystalline alkaloid with a melting point of 194–195° (8).[1] Vinine has been reported to elicit a pronounced and prolonged blood pressure drop in experimental animals (8), whereas reserpinine has been shown to produce

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[1] Pubescine, vinine, and the alkaloid with a melting point of 194–195° were isolated from *Vinca pubescens*, which is considered identical to *Vinca major* var. *major*.

only a transient blood pressure drop (9). The total alkaloids of *Vinca major* have been shown by Quevauviller, *et al.*, to act as ganglioplegics and sympatholytics but do not exert more than a transient drop in blood pressure (10). None of the remaining alkaloids isolated from this plant have been reported to produce any biological activity.

In this paper we wish to report the isolation and properties of perivincine from *Vinca major*. This alkaloid has previously been reported only from *Vinca minor* (11).

EXPERIMENTAL

Extraction of Vinca major Leaves and Stems.—The air-dried ground leaves and stems of *V. major* were packed in a large capacity Soxhlet-type extraction apparatus and extracted with a mixture of 20 volumes of 95% ethanol and 1 volume of 100% methanol for twenty-four hours, or until the leaves and stems were free of alkaloids. The percolate was allowed to cool and was filtered to remove a small amount of nonalkaloidal brown residue. The mixed alcohol-soluble extract was concentrated at 55° under reduced pressure to 10% of its original volume utilizing a continuous model flash evaporator.[1] This concentrate was placed in a refrigerator at 5° overnight and again filtered to remove nonalkaloidal residue.[2]

Isolation of the Total Alkaloids.—One thousand grams of extract was treated with 2,000 ml. of 5% acetic acid which produced a large amount of black resinous material. The mixture was shaken for one hour in a refrigerated room at 5° and then extracted with four separate 500-ml. portions of *n*-hexane at 5° or until the *n*-hexane was no more than faint yellow in color. The *n*-hexane layers were separated, pooled, and the *n*-hexane was removed by distillation. The dark green residue, after drying to constant weight at 60°, represented 5.3% of the air-dried leaves and stems. The *n*-hexane extract was free of alkaloids.

The aqueous acidic extract was kept in a refrigerated room at 5° for twenty-four hours and freed from a small amount of brown residue by filtration. This residue was free of alkaloids and was discarded. The filtrate was concentrated at 55° under reduced pressure to one-third the original volume utilizing a continuous model flash evaporator, then treated with 28% ammonium hydroxide at 5° to a pH of 10 with constant stirring, and the alkaloidal precipitate was removed by filtration. The residue was washed with several portions of distilled water and dried at 60° for twenty-four hours. The filtrate (filtrate A) which was positive for alkaloids was extracted with chloroform. The chloroform extract was dried with anhydrous sodium sulfate, filtered, and the chloroform was removed by distillation. After drying under reduced pressure to constant weight it represented 0.19% of the air-dried leaves and stems.

The alkaloidal residue as removed from the alkaline solution by filtration was very tarry. To further purify this material the residue was taken up in 400 ml. of hot 5% acetic acid, filtered (filtrate B), and reprecipitated with 28% ammonium hydroxide. The alkaloidal precipitate, after washing with distilled water and drying to constant weight, was converted from a black tarry mass to a gray powder which represented 0.12% of the air-dried leaves and stems. The filtrate (filtrate C) was extracted at room temperature with chloroform as described earlier to yield an additional 0.05% of material. The total yield of crude alkaloid was 0.36%.

Isolation and Properties of Perivincine.—The total alkaloids removed by filtration and by extraction of filtrates A and C with chloroform were combined. In a typical experiment, 22 Gm. of the total alkaloids were placed in a suitable flask and refluxed with 200 ml. of benzene for two hours. The benzene solution was cooled to room temperature, and the benzene-soluble material removed from the flask. This procedure was repeated with 200-ml. followed by 100-ml. quantities of benzene, and the benzene extracts were combined and slowly added to 1,000 ml. of petroleum ether (b. p. 30–60°) with constant stirring. A yellow precipitate formed which turned dark brown on standing. Petroleum ether was added until precipitation was complete. The mixture was filtered and the filtrate was concentrated on a water bath to a volume of approximately 15 ml. The concentrate was placed in a refrigerator at 5° for twenty-four hours, at which time small rosette crystals were embedded in a reddish oil. Fifteen milliliters of absolute ether was added and the mixture was stirred until all of the oil and the crystals were dissolved. The solution was allowed to evaporate spontaneously in a dark place, during which time copious amounts of fine needle-like crystals began to form. These were removed by filtration and washed with several portions of ice-cold absolute ethanol. The crude perivincine crystals were dried, weighed, and found to represent from 1.6 to 1.8% of the total alkaloids. Purification of the crude perivincine was effected by several recrystallizations from hot 95% ethanol to a constant melting point.

Anal.[4]—Calcd. for $C_{22}H_{28}N_2O_4$: C, 68.75; H, 7.35; N, 7.29. Found: C, 69.54; H, 7.42; N, 7.37.

The melting point of perivincine determined in a capillary tube (uncorrected), was 199.5–200° (decompn.) and there was no depression when mixed with an authentic specimen of perivincine isolated from *Vinca minor*.[5]

Optical Rotation.—The optical rotation of perivincine was determined by measurement in a Zeiss-Winkel polarimeter with a sample tube length of 1.15 cm. The optical rotation for perivincine was found to be $[\alpha]_D^{25} = -61 \pm 2°$ (pyridine, $c = 0.5$).

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[1] Flash evaporator, continuous model, Laboratory Glass and Instruments Corp., New York 31, N. Y.

[2] An extract prepared in this manner as well as the leaves and stems for the extraction were generously supplied by the S. B. Penick & Co., Inc., 100 Church Street, New York 8, N. Y. The extract as supplied was calculated to be a 44.89% (w/w) concentrate.

[4] Microanalysis of perivincine was conducted by the Analytical Chemistry Section, Research Services of the Mellon Institute, 4400 Fifth Ave., Pittsburgh 13, Pa.

[5] An authentic specimen of perivincine was obtained through the courtesy of Dr. Nathan Rubin, Department of Chemistry, Philadelphia College of Pharmacy and Science, Philadelphia, Pa.

pK Determination.—The pK of perivincine was determined by potentiometric titration of the alkaloids using 75%(v/v) dimethylformamide (Eastman) as the solvent. A Beckman model G pH meter equipped with calomel and glass electrodes was employed for the titration. From the titration data the pK_a of perivincine was calculated to be 8.20.

Ultraviolet and Infrared Spectra.—The ultraviolet absorption spectrum of perivincine was determined at a concentration of 0.01 mg./ml. in 95% ethanol using a Beckman ratio recording spectrophotometer model DK-2. In addition, the infrared absorption spectrum was determined in a Nujol mull, using a Beckman spectrophotometer model IR-4.[6] Both the ultraviolet and infrared data for the perivincine isolated in this study corresponded to the data recorded for perivincine isolated from *Vinca minor* by Scheindlin and Rubin (11).

Screening of Perivincine for Hypotensive Activity.—Perivincine was screened for hypotensive activity in normotensive as well as in hypertensive rats. Albino Wistar rats were made hypertensive by the standard procedure of unilateral nephrectomy one week after a figure-of-eight ligature was placed on the contralateral kidney. The rats were fed a normal diet and administered 0.25 ml. of a desoxycorticosterone trimethylacetate suspension[7] (25 mg./ml.) subcutaneously once a week for ten weeks. A 1% normal saline solution was given in place of drinking water once weekly. At the end of this period of time the rats were anesthetized with urethan and prepared for direct carotid blood pressure recordings according to the method described by Hudak, *et al.* (12).[8]

Perivincine was dissolved in Serpasil placebo solvent[9] (7.5 mg./ml.) and the solution was slowly administered into a femoral vein. Equivalent volumes of solvent were administered intravenously prior to the perivincine solution. A slight transient lowering of blood pressure was noted. Single doses of perivincine ranging from 5 to 20 mg./Kg. were then administered to both normotensive as well as hypertensive rats.

In the rats studied, perivincine produced a rapid transient drop in blood pressure of from 27 to 40%. The return to normal was usually within twenty to thirty minutes. This preliminary screening of perivincine for hypotensive activity indicated that the alkaloid is not a potentially good hypotensive agent.

Paper Chromatography of Perivincine.—The method described by Korzun, *et al.* (13), for the paper chromatographic evaluation of *Rauwolfia* species was modified for the evaluation of *Vinca major* alkaloids. In this method, formamide-saturated paper was used with a mobile phase consisting of equal parts of benzene and cyclohexane, saturated with formamide. Since formamide, as obtained commercially, was found to vary in pH from 10 to 11.7 it was necessary to standardize it in order to obtain reproducible results. This was done by adjusting the formamide with formic acid to various pH levels. It was found that the best resolution of alkaloidal spots was obtained at formamide pH values of 5.2 and 8.0.

Perivincine, dissolved in chloroform, and chromatographed according to this modified method of Korzun, *et al.* (13), exhibited R_f values of 0.08 with formamide adjusted to a pH of 5.2 and R_f 0.17 with formamide adjusted to a pH of 8.0 using a modified Dragendorff's reagent (14) and long wave ultraviolet light to reveal the alkaloidal spots.

The preliminary fractionation of the total alkaloids on alumina columns with subsequent paper chromatographic evaluation of each fraction indicates the presence of at least 37 alkaloids in this plant and the results strongly suggest the presence of tetrahydroalstonine, sarpagine, vincamine, and serpentine. The exact procedure and experimental data for the chromatographic separation of the total alkaloids of *Vinca major* will be published at a later date.

SUMMARY

1. Perivincine has been isolated and identified from the leaves of *Vinca major* L. (*Apocynaceae*).

2. Several analytical constants for this alkaloid have been verified and the optical rotation and pKa determinations have been established.

3. The R_f value of perivincine has been reported in a modified chromatographic procedure at 2 pH values.

4. Preliminary screening of perivincine in anesthetized normotensive and hypertensive rats indicated a rapid, transient drop in blood pressure ranging from 27 to 40 per cent with a return to normal within twenty or thirty minutes.

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[6] Grateful acknowledgment is made to Dr. Hans Noll, Department of Microbiology, School of Medicine, University of Pittsburgh, for performing the infrared study of perivincine.

[7] Generously supplied as Percorten Trimethylacetate intramuscular repository by the Ciba Pharmaceutical Products Inc., Summit, N. J.

[8] The authors wish to thank Mr. Robert K. Bickerton for his assistance in performing the hypotensive screening.

[9] Generously supplied by the Ciba Pharmaceutical Products Inc., Summit, N. J.

A Kinetic Study of Glucose Degradation in Acid Solution*

By KENNETH R. HEIMLICH† and ALFRED N. MARTIN

The degradation of glucose in acid solution was carried out at various temperatures. The first-order rate constants for the decomposition reaction, obtained from the initial rate of formation of 5-hydroxymethylfurfural (5HMF), were consistently higher than those calculated from glucose depletion measurements over long periods of time at temperatures above 100°. At 100° the rate of formation of 5-HMF increased with time for the early portion of the break-down. The rate constant did not coincide with the Arrhenius plot for higher temperatures. This indicates the possibility of a change of mechanism at lower temperatures. The rate data was fitted to kinetic expressions for first-order consecutive reactions. Agreement between calculated and experimental values indicates that the reaction proceeds by the step-wise mechanism. Data obtained from degradation in varying acid concentrations show that the rate constant does not vary linearly with normality. Decomposition in dioxane-water mixtures indicates that the logarithm of the rate constant varies linearly with the reciprocal of the dielectric constant.

A LARGE PORTION of the glucose employed in the pharmaceutical industry is used for the preparation of intravenous solutions. Upon storage these solutions develop a faint straw color, and this instability creates a problem when large quantities of the material are stockpiled for use in times of emergency. Webb, *et al.* (1), carried out a preliminary investigation on the degradation of glucose as part of a project for the Armed Forces. The present study is a continuation of this work with emphasis on the kinetics of the decomposition.

The general scheme of glucose degradation in acid solution has been given by Wolfram, Schuetz, and Cavalieri (2). The decomposition involves the conversion of glucose to 5-hydroxymethylfurfural (abbreviated in this report as 5-HMF) with the subsequent breakdown of this material to formic and levulinic acids and a colored material. It has been indicated by Scallet and Gardner (3) and Joslyn (4) that the color which develops is due to the polymerization of 5-HMF. Saeman (5) studied the breakdown under severe conditions of temperature and acid concentration and also in atmospheres of air, oxygen, and carbon dioxide; he found the depletion to be first order and not affected by the different atmospheres.

Because of the slowness of glucose breakdown at temperatures of 100° and lower, many investigators have used the formation of 5-HMF, which is determined spectrophotometrically, as a criterion for the degradation. Singh, Cantor, and Dean (6) reported that the rate of formation of 5-HMF was indicative of the amount of glucose decomposed. Webb (1) also used the formation of 5-HMF to study the breakdown under varying conditions of catalysis, concentration of glucose, and temperature.

In addition to the degradation to 5-HMF, glucose may undergo a so-called reversion reaction. Many investigators (7–12) have shown this reaction to be manifested as a reversible polymerization condensation to higher saccharides and also the formation of anhydro sugars. It has been stated by Fetzer, *et al.* (9), that the literature on this process is extensive and confusing. Under conditions of low glucose and low acid concentrations the amount of products of this reaction is small.

EXPERIMENTAL

Materials.—Mallinckrodt analytical reagent, anhydrous glucose was used for all tests, and the solutions were prepared with deionized, distilled water. The 5-HMF was obtained from the Bios Laboratories (17 W. 60th St., New York, N. Y.). It was purified by vacuum distillation at a reduced pressure of 2 mm. Hg. The dioxane used was Eastman's analytical grade, and was purified according to the method given by Kraus and Vingee (13). All other chemicals used were of the highest purity available, usually analytical reagent or reagent grade. The decomposition of the solutions was carried out in 5-ml. Tuf-Top Neutraglas ampuls obtained from the Kimble Glass Co., Toledo, Ohio.

Apparatus.—The constant temperature source was a Bayley model 118 bath (Bayley Instrument Co., Danville, Calif.). The bath has a stated precision of ±0.001°. The 5-HMF concentrations were determined on a Beckman DU spectrophotometer with the ultraviolet attachment. The solutions

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were read in fused silica cells. The pH determinations were made on a Beckman model G pH meter.

Assay.—The degradation of the glucose solutions was followed either by the formation of 5-HMF or the depletion of glucose, and in some cases both methods were employed on the same solution. The determination of 5-HMF was done spectrophotometrically. The solutions were filtered to remove any insoluble humus material, diluted, and read against a glucose blank of the proper concentration at a wavelength of 284 mμ. The absorption readings were converted to moles per liter by the expression

$$C = A \times 6.298 \times 10^{-5} \qquad \text{(Eq. 1)}$$

where A is the absorbance of the solution. This equation was obtained from the standard curve of a Beer plot.

The glucose was determined by an iodometric method first used by Romijn (14) and further tested by Cajori (15). The method consists of reacting the solutions with a measured excess of iodine and titrating the residual iodine with standard thiosulfate solution. The equation for converting to the amount of glucose present is

$$\text{moles/liter of glucose} = (R - S) \times N \times 0.1 \qquad \text{(Eq. 2)}$$

where N is the normality of the thiosulfate solution, R is the milliliters of thiosulfate required to react with an iodine blank, and S the amount required to react with the sample. The accuracy of the method was determined to be from 1 to 3% in the presence of decomposition products of the reaction.

RESULTS AND DISCUSSION

The decomposition of 0.056 molar glucose solutions in 0.35 N hydrochloric acid was followed by glucose depletion and by 5-HMF formation from 100 to 150°. Plots of the logarithm of the amount of glucose remaining against time produced good linearity which indicates a first-order decomposition. The rate constants were calculated from

$$k_1 = (2.303/t) \log (A_0/A) \qquad \text{(Eq. 3)}$$

where A is the concentration of glucose in moles/liter at any time, t is the time in hours, A_0 is the original concentration of glucose in moles/liter, and k_1 is the first-order rate constant having the unit of reciprocal time. These values are given in Table I.

TABLE I.—FIRST-ORDER RATE CONSTANTS FOR GLUCOSE BREAKDOWN IN 0.35 N HYDROCHLORIC ACID

Temperature, °C.	Rate Constant, k_1 hr.$^{-1}$
100	0.0012
110	0.0040
120	0.0120
130	0.0267
140	0.0628
150	0.1693

Under the conditions used in this study the induction type curve for 5-HMF formation reported by Webb, *et al.* (1), was evidenced only at 100°.

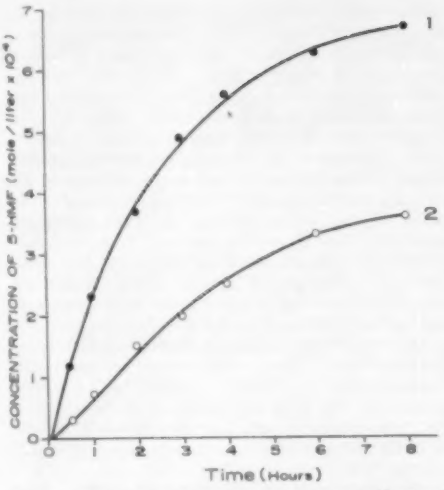


Fig. 1.—The formation of 5-hydroxymethylfurfural at 110° (curve 1) and at 100° (curve 2).

The changing nature of the curve with time and temperature is shown in Fig. 1. Rate constants based on 5-HMF formation were obtained from plots of this nature by calculating the initial slope, $(dB/dt)_i$. These values were obtained by placing a mirror on the curve perpendicular to the plane of the paper and adjusting it until the image of the curve produced a smooth, continuous arc with the curve itself. The slope of a line perpendicular to the mirror as read from the plot at zero time yielded the desired value. Assuming that the formation of 5-HMF is a measure of glucose depletion, and disregarding the possible consecutive steps of the reaction at this point (*cf.* Eq. 6) the following expression may be written

$$(dB/dt)_i = k_i A \qquad \text{(Eq. 4)}$$

where $(dB/dt)_i$ is the initial slope of the formation curve, k_i is the first-order rate constant, and A is the concentration of glucose. In order to obtain the rate constant the equation is rearranged to

$$k_i = (dB/dt)_i/A \qquad \text{(Eq. 5)}$$

The subscript i is used in the k_i since the rate constant is based on the initial rate. The values of k_i from 100 to 140° are given in Table II.

A comparison of Tables I and II shows that the values of k_1 are consistently smaller than the values of k_i except at 100°. It is important to point out

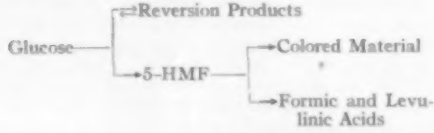
TABLE II.—VALUES OF k_i OBTAINED FROM 5-HMF FORMATION FOR GLUCOSE BREAKDOWN IN 0.35 N HYDROCHLORIC ACID

Temperature, °C.	k_i, hr.$^{-1}$
100	0.0009
110	0.0054
120	0.0179
130	0.0330
140	0.1063

this difference in the rate constants obtained by the two different methods, since glucose decomposition studies in the literature have been based on one or the other of these criteria. Some authors have calculated the rate constant from the breakdown at some arbitrary time rather than initially. The present study shows that this procedure is unsatisfactory because of the changing slope of the 5-HMF curve. Since the initial rate is the velocity of the reaction before any interference from products can occur, k_i may be accepted as the more significant quantity. The k_1 values, obtained from the integrated equation, are based on the degradation reaction over a long period of time. Even though the reaction follows the integral order with respect to the method of integration it is possible that products of the reaction are interfering, giving k_1 values which are lower than the k_i values based on the initial formation of the 5-HMF. Laidler (16) has also pointed to the possible discrepancy between these two rate constants and has recommended the use of k_i for complex reactions such as involved in the present study.

The logarithm of the rate constants, k_1 and k_i, were plotted against the reciprocal of the absolute temperature according to the Arrhenius equation and are shown in Fig. 2. The activation energy calculated from k_i values was 31.8 Kcal./mole which compares favorably to that of 31.2 Kcal./mole obtained from k_1 values.

Application of the Rate Data to a Kinetic Expression.—From the literature (2–4, 11, 17) and from our own observation, the degradation of glucose in acid solution may be represented by the scheme



The reversion reaction is reversible, and at low glucose and acid concentrations the formation of reversion products is slight and may be neglected here. Due to the indefinite nature of the 5-HMF breakdown products, the expression for the degradation of glucose may be written simply as

$$a \xrightarrow{k_1} b \xrightarrow{k_2} c \qquad \text{(Eq. 6)}$$

where a is glucose, b is 5-HMF, c is the breakdown products of 5-HMF, and k_1 and k_2 are the first-order rate constants. A consecutive reaction such as this was studied by Harcourt and Esson (18), and the mathematical expressions were derived by Esson (19). If A_0 is the initial concentration of a, and A, B, and C are the concentrations of a, b, and c, respectively, at any time, t, then

$$A_0 = A + B + C \qquad \text{(Eq. 7)}$$

The rate of disappearance of a is given at any instant by

$$-dA/dt = k_1 A \qquad \text{(Eq. 8)}$$

which yields upon integration

$$A = A_0 e^{-k_1 t} \qquad \text{(Eq. 9)}$$

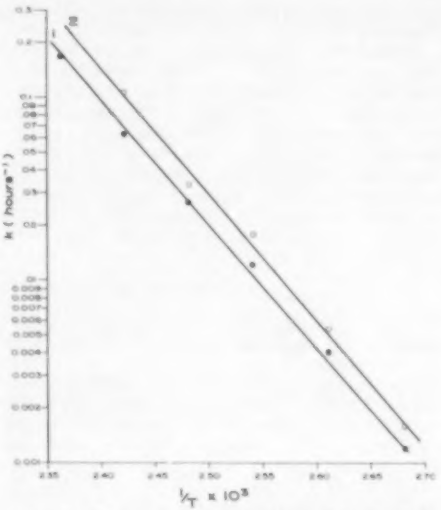


Fig. 2.—Temperature dependence of k_1, the first-order rate constant based on glucose depletion (curve 1), and k_i, the first-order rate constant based on the initial formation of 5-hydroxymethylfurfural (curve 2).

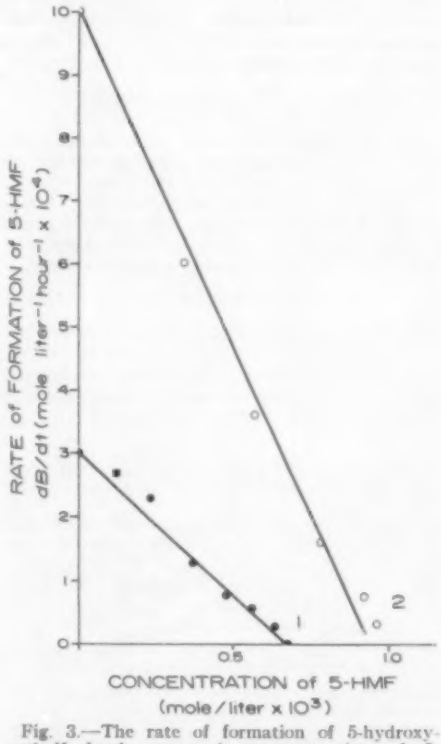


Fig. 3.—The rate of formation of 5-hydroxymethylfurfural *versus* the concentration of 5-hydroxymethylfurfural at 110° (curve 1) and 120° (curve 2).

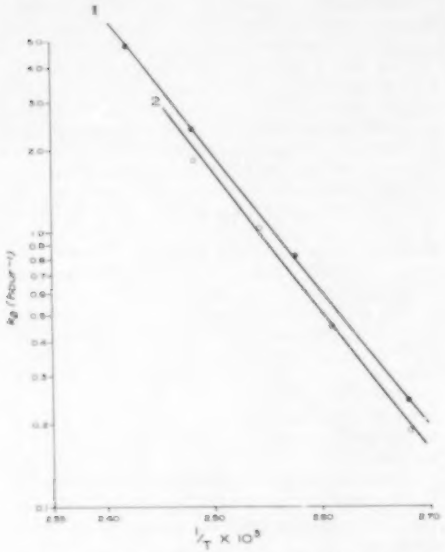


Fig. 4.—Temperature dependence of the experimental rate constant (curve 1) and the calculated rate constant (curve 2) for 5-hydroxymethylfurfural degradation.

The rate at which the concentration of b increases is given by

$$dB/dt = k_1A - k_2B \qquad \text{(Eq. 10)}$$

which upon integration and substitution of A from Eq. 9 becomes

$$B = \frac{A_0k_1}{k_2 - k_1}(e^{-k_1t} - e^{k_2t}) \qquad \text{(Eq. 11)}$$

The formation of c is given by the expression

$$dC/dt = k_2B \qquad \text{(Eq. 12)}$$

Upon integration and the substitution of B from Eq. 11 the following is obtained

$$C = A_0\left[1 + \frac{1}{k_1 - k_2}(k_2e^{-k_1t} - k_1e^{-k_2t})\right] \qquad \text{(Eq. 13)}$$

The value of C may also be obtained from Eq. 7 by difference.

The data can be fitted to the kinetic expression by calculating the values of k_2 by a graphical method employing Eq. 10. The left hand side of the equation represents the rate at which 5-HMF is forming at any time, t. The values of dB/dt were obtained by taking tangents from the plot of B *versus* t using a mirror as described earlier. These values of dB/dt were plotted against B. The result expected is a straight line plot, the slope of which is $-k_2$ and whose intercept is k_1A, where k_1 is used instead of k_1 for the reason previously given. Plots of this nature for 110 and 120° are shown in Fig. 3. Good linearity was found for the earlier portions of the breakdown in each case. The slopes of the plots were taken to obtain the values of k_2. A comparison of k_2 values calculated in this manner using Eq. 10, and k_2 values obtained experimentally from the degradation of pure 5-HMF in the same acid concentration, is shown in Table III. The activation energies were obtained from the Arrhenius plots for each set of constants shown in Fig. 4. The activation energy obtained from the calculated values of k_2 was 23.4 Kcal./mole while that from the experimental values of k_2 was 22.7 Kcal./mole.

TABLE III.—A COMPARISON OF CALCULATED AND EXPERIMENTAL VALUES OF k_2

Temperature, °C.	k_2 Hr.$^{-1}$, Calcd.	k_2 Hr.$^{-1}$, Exptl.
100	0.1875	0.2434
110	0.4533	0.5450
120	1.0500	1.2100
130	1.8400	2.4048

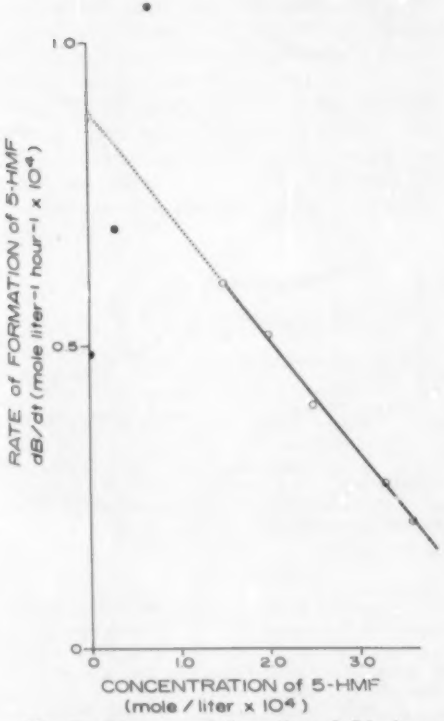


Fig. 5.—The rate of formation of 5-hydroxymethylfurfural *versus* the concentration of 5-hydroxymethylfurfural at 100°. Solid points show initial increase in rate at low concentrations. Extrapolation yields rate consistent with higher temperatures.

A plot of dB/dt (the rate of formation of 5-HMF) *versus* B (the concentration of 5-HMF) at 100° is shown in Fig. 5. The first 3 points on the plot indicate the increasing value of dB/dt as depicted by the increase in the slope of the initial portion of curve 2 in Fig. 1. The extrapolation of the linear portion of the curve in Fig. 5 to $B = 0$ yields a value of k_1A according to Eq. 10. The value obtained by

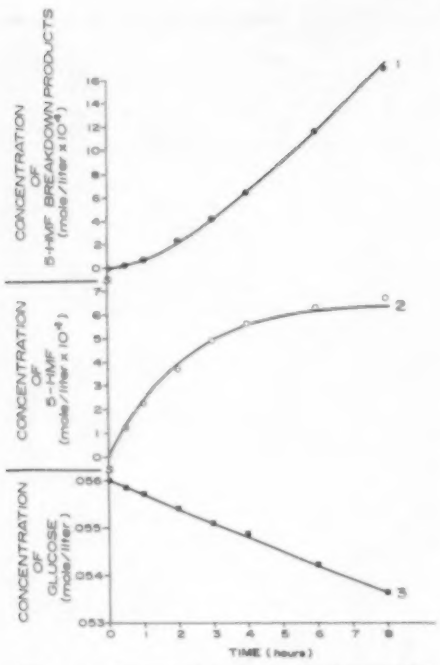


Fig. 6.—Concentration of 5-hydroxymethylfurfural breakdown products (curve 1), 5-hydroxymethylfurfural (curve 2), and glucose (curve 3), at various times at 110°.

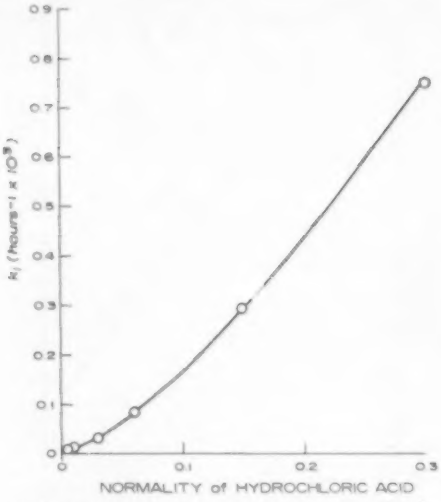


Fig. 7.—Variation of the rate constant (k_1) for glucose degradation with the normality of hydrochloric acid.

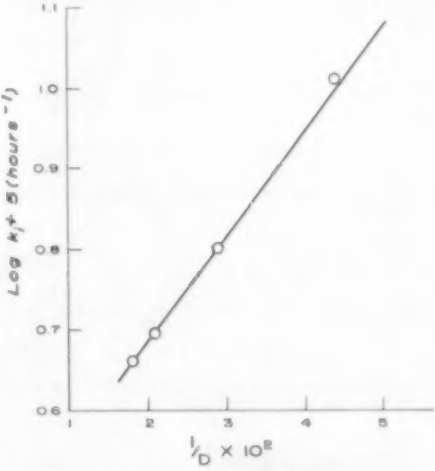


Fig. 8.—Variation of the logarithm of the rate constant (k_1) for glucose degradation with the reciprocal of the dielectric constant.

this method for k_1 was 0.0016 hr.$^{-1}$ as compared to 0.0009 hr.$^{-1}$ given in Table II. The former value is consistent with the Arrhenius plot for k_1 values at higher temperatures given in Fig. 2 (curve 2). Since the later value is not consistent with the plot, the possibility exists that at 100° and below the formation of 5-HMF may proceed by a different mechanism. It also points out dramatically the pitfalls one may encounter in stability testing when the Arrhenius plot is extrapolated from higher to lower temperatures. The activation energy in this study (31.8 Kcal./mole) compares favorably to that found by Webb, *et al.* (1), at lower temperatures (31.0 Kcal./mole). Thus, even though the activation energies for the different temperature ranges are similar, extrapolation of the plot from higher temperatures would yield higher rate constants than the actual values.

A further check on the kinetic expressions was obtained by employing the integrated Eqs. 11 and 13. Using k_1 as the assumed true rate constant for glucose breakdown and the calculated values of k_2, it is possible to calculate the amounts of 5-HMF and 5-HMF breakdown products present. These values at 110° are represented by the curves in Fig. 6. The points of these plots indicate the actual experimental values. Agreement between the calculated and experimental results lends support to the idea that the reaction proceeds by a stepwise mechanism represented by the simplified scheme of Eq. 6. The top diagram of Fig. 6 is the important one for the breakdown of glucose solutions because it shows the rate of formation of the colored compounds. Some workers have used the rate of formation of 5-HMF (center diagram of Fig. 6) as a measure of the rate of discoloration, but it can be seen here that the shape of the curves are not alike. The 5-HMF formed from glucose is an intermediate in the production of colored compounds, and its rate of formation actually falls off as more colored material (humus) is formed.

Influence of Acid on Glucose Breakdown.—Degradation of 0.116 molar glucose solutions was carried out in varying concentrations of hydrochloric acid at a constant ionic strength and at 100°. The initial rate constant, k_i, was plotted against the normality in Fig. 7, and it is seen that the relationship is not linear. This makes the extrapolation to zero acid concentration to obtain the spontaneous rate somewhat inaccurate. However, at the very low acid concentrations the curve does assume a nearly linear form, which was also reported by Webb, *et al.* (1).

Influence of the Dielectric Constant on the Rate Constant.—The degradation of 0.278 molar glucose solutions at a pH of 1.27 and a temperature of 100° was carried out in dioxane-water mixtures. The dielectric constants of the solutions were calculated from data collected by Akerlof and Short (20). As a first approximation the dielectric constant of the solution was assumed to be that of the solvent. A plot of the logarithm of the initial rate constant *versus* the reciprocal of the dielectric constant is shown in Fig. 8. A straight line of positive slope is evidenced, a result that is predicted by theory for a reaction between a positive ion and a dipole molecule (21).

SUMMARY

1. The degradation of glucose in acid solution was carried out at various temperatures. It was found that the rate constants calculated from the measurement of 5-HMF formation were consistently higher than those calculated from glucose depletion.

2. The rate data were fitted to a kinetic expression for first-order consecutive reactions. Good agreement between calculated and experimental values indicated that the reaction, although no doubt more complicated, can be represented satisfactorily as proceeding by this simplified scheme.

3. The rate constant at 100° was not consistent with the values found at higher temperatures. A possible change in mechanism is indicated at the lower temperatures.

4. The relationship between the rate constant and acid concentration was found not to be linear.

5. Studies in solutions of varying dielectric strength indicate that the degradation involves a reaction between a positive ion and a dipole molecule.

6. The results of this study suggest that the discoloration of glucose solutions under the conditions employed (acid solutions at elevated temperatures) follows from the breakdown of glucose and proceeds through the intermediate formation and breakdown of 5-hydroxymethylfurfural. The rate constants obtained at elevated temperatures can not be extrapolated to obtain the rate of breakdown at room temperature because of a possible difference in mechanism.

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Theoretical Analysis of Diffusional Movement Through Heterogeneous Barriers*

By W. I. HIGUCHI and TAKERU HIGUCHI

A number of theoretical relations dealing with diffusion through heterogeneous barriers are presented and discussed. These are expected to be useful in predicting barrier behavior in formulations such as pharmaceutical and particularly protective ointments and films. A relationship has been derived which expresses the effective permeability constant, P_m, of a two phase mixture as a function of the volume fraction and the permeability of each phase. The effect of the shape of the particles on P_m is also considered. Where data are available, agreement with theory is satisfactory. The nonstationary state behavior of a two phase heterogeneous barrier is discussed in relation to the diffusion coefficient, partition coefficient, the volume fraction, and the particle size associated with each phase. It is shown that the effectiveness of one heterogeneous barrier may be greater than another for a given time of exposure while for a longer time of exposure the reverse may be the case. The effect of a "skin" or a coat of a third phase on the internal phase particle has also been examined. Finally, the situation in which simultaneous diffusion and absorption occur is discussed and an expression for the lag time has been derived for this case.

THE PURPOSE of this study is to present some theories and relationships of pharmaceutical and pharmacological importance which deal with diffusional movement of chemical molecules through heterogeneous systems where only volume molecular diffusion is important. Although extensive experimental and theoretical studies (1) have been carried out on permeation of gases and vapors through various materials, these studies have been primarily restricted to plastic films. Diffusion in emulsions, suspensions, and powders has received relatively little attention.

Since the phenomenon of diffusion in heterogeneous materials is of great importance to workers in the fields of pharmacy and pharmacology, it was thought of interest to prepare a rather detailed theoretical analysis of this very significant mode of mass transfer. As far as we know much of what is presented here is new, representing in part an extension of a report made to the U. S. Army Chemical Corps (2).

In the majority of practical cases encountered in pharmaceutical and pharmacological areas the diffusion barrier is of heterogeneous rather than homogeneous nature. Thus the present subject is of importance in the following particular problems: (*a*) the design of protective ointments and films which can be employed against toxic and corrosive agents, (*b*) the study of drug release from pharmaceutical ointments and orally administered barrier coated dosage forms, and (*c*) the study of the absorption of drugs through skin and other passive biological membranes.

The aspect of this subject which was of particular interest to this laboratory was in connection with the understanding of the protective action of ointments (3). It is intended that the present report provide a clarification of the important variables rather than a rigorous mathematical treatment of this problem. The latter is impossibly difficult in many cases due to the complexity of the picture. It is assumed that the reader is familiar with the fundamentals of the diffusion problem. Material which is adequately covered by standard texts on diffusion such as those by Jost and Barrer (1), is not reviewed here but is only referred to when needed. This applies primarily to the understanding of Fick's law of diffusion and its application.

In this report the discussion will be in two parts. The first section will deal with steady state diffusion while the second part will treat the nonstationary state aspects of diffusion. As it will be seen, the effectiveness of a heterogeneous barrier predicted by a steady state analysis is not necessarily a measure of its time-dependent qualities.

STEADY STATE DIFFUSION THROUGH EMULSION AND SUSPENSION BARRIERS

For the discussion the diagram given in Fig. 1 will be followed. On the left side of the barrier of thickness h and cross sectional area of unity is a well stirred solution of the penetrating agent at a constant concentration $C = C_a$, activity $a = a_a$ (referred to the pure liquid agent as unity),[1] and activity coefficient γ_a. On the right side of the barrier is the solution of the agent $a = a_b$. It is assumed that $h \ll$

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[1] Following the Hildebrand convention, we take activity = 1 for the pure liquid (or supercooled liquid). Then K_a is equal to the ratio of the activity coefficients. This convention is preferable to any other for our purposes.

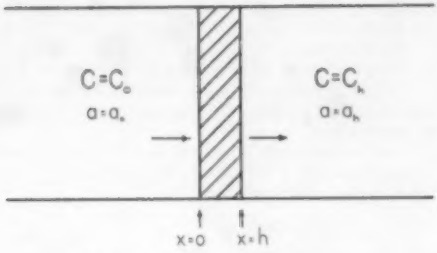

Fig. 1.—Diffusion through barrier.

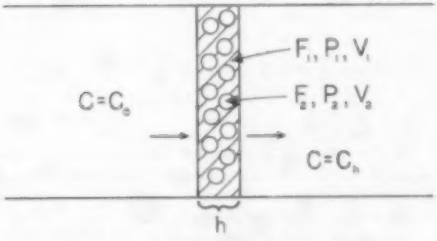

Fig. 2.—A two-phase diffusion barrier.

1 and that both Henry's law and Fick's law are obeyed throughout the barrier.

If the barrier is homogeneous and if in the barrier the diffusion coefficient and the activity coefficient are D and γ, respectively, the rate of permeation for the case of similar solvents on both sides of the barrier is

$$p = \frac{D}{\gamma}\frac{da}{dx} = \frac{D\gamma_o(C_o - C_h)}{\gamma h} = \frac{DK_a(C_o - C_h)}{h} \qquad \text{(Eq. 1a)}$$

where K_a is the partition coefficient[1] of the agent for the two media. The product DK_a may be set equal to

$$DK_a = P$$

where P is the permeability constant for the system.

For the more general case of the solvents differing on both sides of the barrier, the rate of permeation would be

$$p = \frac{P_oC_o + P_hC_h}{h} \qquad \text{(Eq. 1b)}$$

where P_o and P_h refer to the two solvents.

General Case.—The rigorous mathematics for the steady state diffusion problem for a barrier composed of one or more phases randomly or otherwise distributed in another phase is not available nor is it readily derivable. Following the analogous electrostatic problem (4–6) it is possible, however, to obtain approximate relationships between P_m, the effective permeability constant of the barrier, and permeability constants and the volume fractions of the individual phases. In what follows, expressions for P_m for random suspensions of spheres and spheroids are derived following the method of one of the authors (5) based on dielectric constant data of powders and suspensions.

Figure 2 is a schematic illustration of the model to be used. For simplicity, only the case of a mixture of two isotropic phases will be treated, although the model may be easily extended to the general case of many phases. Also, it is assumed that the particles of the internal phase are uniform in shape and size, immobile in the barrier, small compared to the thickness of the barrier, but large enough to be considered phases. The barrier, then, is composed of an external phase, 1, and an internal phase, 2. The respective agent activity coefficients and diffusion coefficients are γ_1, γ_2, D_1, and D_2 in the two phases. The average, i. e., the macroscopic, activity gradient of the agent in the barrier, the thickness of which is small compared to its volume, is of the magnitude

$$F_o = \frac{a_o - a_h}{h}$$

and is directed in the x-direction. The rate of steady state permeation is then

$$p = \frac{D_m}{h\,\gamma_m}(a_o - a_h) = P_mF_o$$

where D_m and γ_m are the effective diffusion coefficient and the effective activity coefficient of the penetrating agent with respect to the barrier. Boundary layer diffusion will be neglected and it will be assumed that the rate of interphase transport of the agent at the boundaries is rapid compared to volume diffusion.

In any macroscopic portion of the barrier one finds that the following is true:

$$\frac{D_mF_o}{\gamma_m} = \frac{D_1}{\gamma_1}V_1F_1 + \frac{D_2}{\gamma_2}V_2F_2$$

or

$$P_mF = P_1V_1F_1 + P_2V_2F_2 \qquad \text{(Eq. 2)}$$

where F_1 and F_2 are the average activity gradients of the penetrant in phase 1 and phase 2, respectively, and V_1 and V_2 are the respective volume fractions of the two phases. Vectorially, F_1 and F_2 are directed in the same direction as F_o, *viz.* in the x-direction. Equation 2 states that the rate of permeation of the agent in the barrier is equal to the sum of the rates through the two phases. Also by definition, the following is true:

$$F_o = V_1F_1 + V_2F_2 \qquad \text{(Eq. 3)}$$

It is worth while to emphasize at this point that in the development of the theory it is the activity rather than the concentration of the agent which is the driving force or the potential for diffusion; it is the activity gradient rather than the concentration gradient which is the field. A solute will diffuse from a region of low concentration to a region of high concentration if the activity of the solute is less in the high concentration region.

Internal Phase Consisting of Random Spheres.—If the internal phase is composed of a system of randomly distributed uniform isotropic spheres and if it is assumed that each sphere is placed in a parallel and uniform activity gradient, F_1, then according to the solution of LaPlace's equation with the proper boundary conditions (5)

$$F_2 = \left(\frac{3P_1}{2P_1 + P_2}\right)F_1 \qquad \text{(Eq. 4)}$$

However, Eq. 4 is true for a suspension only when P_2/P_1 is near unity or when V_2 is near zero. A more accurate approach is to assume that the particle is placed in an activity gradient

$$F_1' = F_1 + \Delta F_1 \qquad \text{(Eq. 5)}$$

where ΔF_1 is the contribution due to the interaction of the neighboring particles with the particle in question. The meaning of ΔF_1 may be presented in the following manner: a particle, say i, reacts to the field $F_1' = F_1 + \Delta F_1$ and creates a perturbation field of its own. The neighboring particles react to this perturbation and create the excess field ΔF_1 at i. It was shown (5) that ΔF_1 has approximately the form

$$\Delta F_1 = \frac{\alpha^3 K F_1'}{\bar{a}^3} \qquad \text{(Eq. 6)}$$

for spheres where $\bar{a}$ is the particle radius and

$$\alpha = \left(\frac{P_2 - P_1}{2P_1 + P_2}\right)\bar{a}^3$$

Then the more accurate expression for F_2 is given by

$$F_2 = \left[\frac{3P_1}{2P_1 + P_2}\right]\left[\frac{1}{1 - K\left(\frac{P_2 - P_1}{2P_1 + P_2}\right)^3}\right] \qquad \text{(Eq. 7)}$$

The quantity K involves the distribution function for random spheres and is a function of V_2. It is independent of P_1 or P_2 if, in the derivation of Eq. 6, the terms in α higher than α^3 could be neglected.

Then, if Eqs. 7, 3, and 2 are solved for P_m

$$P_m = \frac{2P_1^2(1 - V_2) + P_1P_2(1 + 2V_2) - KP_1\left(\frac{P_2 - P_1}{2P_1 + P_2}\right)^3 (2P_1 + P_2)(1 - V_2)}{P_1(2 + V_2) + P_2(1 - V_2) - K\left(\frac{P_2 - P_1}{2P_1 + P_2}\right)^3 (2P_1 + P_2)(1 - V_2)} \qquad \text{(Eq. 8)}$$

Had Eq. 4 been used instead of Eq. 7, the same expression would have resulted, but with $K = 0$, *viz.*, an expression analogous to the Maxwell-Rayleigh-Lorentz-Clausius-Mosotti equations (6).

Physically the difference between Eqs. 4 and 7 may be summarized as follows: Eq. 4 assumes that the induced activity gradient, F_2, of the internal phase particle in question is due wholly to the perturbation caused by the particle in question to the field, F_1. Recall, however, that F_1 is the result of the presence of all the other internal phase particles as well as the presence of the external phase. On the other hand, Eq. 7 assumes that the induced activity gradient, F_2, of the particle in question must also include the effect of the modification of F_1 due to the response of nearby internal phase particles to the "initial" response of the particle in question to F_1. This latter is analogous to an induced-induced-induced effect in electrostatics.

Internal Phase Consisting of Random Spheroids.—In order to be able to estimate the effects of shape of the particles, the same treatment is carried out for randomly distributed and randomly oriented spheroids.

The first step is to evaluate ΔF_1, given by Eq. 6. As information is lacking, it will be assumed that the translational distribution function for randomly distributed ellipsoids is the same as that for a system of spheres with the same V_2.

The perturbation potential outside an ellipsoid oriented with its a-semiaxis in the direction of a uniform and parallel field F_1' is (7)

$$\phi = \frac{1}{2}\left[\frac{\bar{a}\bar{b}\bar{c}\left(\frac{P_2 - P_1}{P_1}\right)\int_s^\infty \frac{ds}{(s + \bar{a}^2)R_s}}{1 + \frac{\bar{a}\bar{b}\bar{c}}{2}\left(\frac{P_2 - P_1}{P_1}\right)\int_o^\infty \frac{ds}{(s + \bar{a}^2)R_s}}\right] F_1' x \qquad \text{(Eq. 9)}$$

where F_1' is directed along the x-axis

$$R_s = [(s + \bar{a}^2)(s + \bar{b}^2)(s + \bar{c}^2)]^{1/2}$$

and $\bar{b}$ and $\bar{c}$ are the other two semiaxes.

If the ellipsoid is oriented with a different axis parallel to the field, an expression identical to Eq. 9 is obtained except that the appropriate semiaxis value is exchanged for $\bar{a}$ in Eq. 9.

At distances large compared to the largest semiaxis the perturbation potential becomes (8)

$$\phi = \frac{2}{3}\left[\frac{\bar{a}\bar{b}\bar{c}\,(P_2 - P_1)}{2P_1 + \bar{a}\bar{b}\bar{c}(P_2 - P_1)\int_o^\infty \frac{ds}{(\bar{a}^2 + s)R_s}}\right]\frac{F_1'}{r^3} x$$

where r is the distance to the point in question from the center of the ellipsoid.

This may be written

$$\phi = \frac{\alpha F_1'}{r^3} x$$

where

$$\alpha = \frac{1}{3}\left[\frac{\bar{a}\bar{b}\bar{c}\,(P_2 - P_1)}{\frac{\bar{a}\bar{b}\bar{c}}{2}(P_2 - P_1)\int_o^\infty \frac{ds}{(\bar{a}^2 + s)R_s}}\right]$$

If the ellipsoid is randomly oriented with respect to the field F_1' with equal probability of orientations in all directions

$$\alpha = \frac{1}{9}\bar{a}\bar{b}\bar{c}(P_2 - P_1)\left[\frac{1}{P_1 + (P_2 - P_1)A_{\bar{a}}} + \frac{1}{P_1 + (P_2 - P_1)A_{\bar{b}}} + \frac{1}{P_1 + (P_2 - P_1)A_{\bar{c}}}\right] \qquad \text{(Eq. 10)}$$

where

$$A_{\bar{a}} = \frac{1}{2}\bar{a}\bar{b}\bar{c}\int_o^\infty \frac{ds}{(\bar{a}^2 + s)R_s}$$

and for spheroids

$$A_{\bar{b}} = A_{\bar{c}} = \frac{1}{2}(1 - A_{\bar{a}})$$

Equation 10 for α is only strictly valid at large r. However it is expected to be approximately correct

for not too large r. When $\bar{a} = \bar{b} = \bar{c}$, Eq. 10 reduces to the α expression for the sphere, *viz.*

$$\alpha = \left[\frac{(P_2 - P_1)}{(2P_1 + P_2)}\right]\bar{a}^3$$

The expression for ΔF_1 is then (5, 6)

$$\Delta F_1 = \frac{\dfrac{\alpha^3 K}{(\bar{a}\bar{b}\bar{c})^2}}{1 - \dfrac{\alpha^3 K}{(\bar{a}\bar{b}\bar{c})^2}} F_1 \qquad \text{(Eq. 11)}$$

The expression for F_2 for randomly oriented ellipsoids in a uniform and parallel field F_1' is (9)

$$F_2 = \frac{1}{3} F_1' P_1 \left[\frac{1}{P_1 + (P_2 - P_1)A_{\bar{a}}} + \frac{1}{P_1 + (P_2 - P_1)A_{\bar{b}}} + \frac{1}{P_1 + (P_2 - P_1)A_{\bar{c}}}\right]$$

or

$$F_2 = \frac{1}{3} F_1 P_1 \left[\frac{1}{1 - \dfrac{\alpha^3 K}{(\bar{a}\bar{b}\bar{c})^2}}\right]\left[\frac{1}{P_1 + (P_2 - P_1)A_{\bar{a}}} + \frac{1}{P_1 + (P_2 - P_1)A_{\bar{b}}} + \frac{1}{P_1 + (P_2 - P_1)A_{\bar{c}}}\right] \qquad \text{(Eq. 12)}$$

Now if Eqs. 2, 3, and 12 are solved for P_m with $\bar{a} \neq \bar{b} = \bar{c}$, i.e., for spheroids,

$$P_m = \frac{P_1 V_1 + P_2 V_2 L}{V_1 + V_2 L} \qquad \text{(Eq. 13)}$$

where

$$L = \frac{P_1}{3}\left[\frac{1}{1 - \dfrac{\alpha^3 K}{(\bar{a}\bar{b}\bar{c})^2}}\right]\left[\frac{1}{P_1 + (P_2 - P_1)A_{\bar{a}}} + \frac{2}{P_1 + (P_2 - P_1)A_{\bar{b}=\bar{c}}}\right]$$

and

$$\alpha = \frac{1}{9}\bar{a}\bar{b}\bar{c}\,(P_2 - P_1)\left[\frac{1}{P_1 + (P_2 - P_1)A_{\bar{a}}} + \frac{2}{P_1 + (P_2 - P_1)A_{\bar{b}=\bar{c}}}\right]$$

For spheroids the expressions for $A_{\bar{a}}$ and $A_{\bar{b}} = A_{\bar{c}}$ are simple (10): $A_{\bar{a}} = \dfrac{1}{1 - \mathrm{Z}^2} - \dfrac{\mathrm{Z}}{(1 - \mathrm{Z}^2)^{3/2}} \cos^{-1} \mathrm{Z}$ for $\bar{a} < \bar{b} = \bar{c}$ and $\mathrm{Z} = \bar{a}/\bar{b}$; $A_{\bar{a}} = -\dfrac{1}{\mathrm{Z}^2 - 1} + \dfrac{\mathrm{Z}}{(\mathrm{Z}^2 - 1)^{3/2}} \ln\left[\mathrm{Z} + (\mathrm{Z}^2 - 1)^{1/2}\right]$ for $\bar{a} > \bar{b} = \bar{c}$ and $\mathrm{Z} = \bar{a}/\bar{b}$. In Table I some values for $A_{\bar{a}}$ and $A_{\bar{b}} = A_{\bar{c}}$ are given.

TABLE I.—CALCULATED VALUES OF A FOR SPHEROIDS

Z	$A_{\bar{a}}$	$A_{\bar{b}} = A_{\bar{c}}$
10	0.020	0.490
5	0.059	0.471
2	0.173	0.413
0.5	0.531	0.235
0.2	0.750	0.125
0.1	0.860	0.070
0.01	0.984	0.008

Comparison of Theoretical Predictions With Experimental Data.—Unfortunately, diffusion data over a wide range of P_2 and P_1 do not appear to be available in the literature. However, the analysis (5) of dielectric constant data for powders and suspensions over a wide range demonstrated that Eq. 8 with $K = 0.78$ is a satisfactory representation of the effective dielectric constant of a two-phase mixture in which the particle shapes of the internal phase are not too different from spherical. As the mathematics for steady state dielectric polarization and for steady state one component diffusion are identical as long as saturation effects and interfacial effects are negligible, the conclusions regarding one problem should apply to the other.

Although, according to the theory, K was expected to be a function of V_2, fortuitous circumstances (5) made necessary a choice of only a single value of K used with Eq. 8 to permit the expression to be applicable over a wide range of P_2, P_1, and V_2. The value of 0.78 for K was expected to absorb asphericity effects arising from actual particle shape irregularities and from particle-particle contacts in the system. The K for true spheres accordingly was expected to be smaller than 0.78.

Most of the available diffusion data for heterogeneous systems in the literature apply only to the extreme case of $P_2 = 0$, i.e., zero permeability for the internal phase. In Fig. 3 the selected data of Carman (11) for $P_2 = 0$ are plotted. The quantity q plotted abainst V_1, the porosity, is defined as $q = V_1 P_1 / P_m$.

The data included experiments on electrical conduction as well as diffusion. Also given in Fig. 3 are curves predicted by available theoretical formulas for mixtures. Curve A is the Maxwell-Clausius Mosotti formula for a suspension of spheres or Eq. 8 with $K = 0$. Curve B is Eq. 8 with $K = 0.78$. Curve C is Bruggeman's formula for random spheres (4), *viz.*

$$\frac{P_2 - P_m}{P_2 - P_1} = V_1 \left(\frac{P_m}{P_1}\right)^{1/3} \qquad \text{(Eq. 14)}$$

which, when $P_2 = 0$, becomes $P_m = P_1 V_1^{3/2}$. Curves D and E are plots of Eq. 13 with $K = 0.78$ for spheroids with axial ratios $\mathrm{Z} = 2.0$ and $\mathrm{Z} = 0.5$, respectively. Equation 13 must be regarded as one which can give only a rough prediction of the effects of shape since both the Eq. 12 for α and the idea that the same K for spheres may be used for spheroids are approximate. It is expected to be useful when Z is not too different from unity.

Although the scatter in the data is large, the data for spheres (circles in Fig. 3) appear to be localized in one area. A K value somewhat smaller than 0.78 used with Eq. 8 will give satisfactory agreement with the data for spheres. This is in agreement with the idea (5) that $K = 0.78$ does absorb an asphericity factor. The experimental lower limit for K appears to be somewhat near 0.4 to 0.5 with these data. The data for systems involving random nonspherical particles always give higher q values than

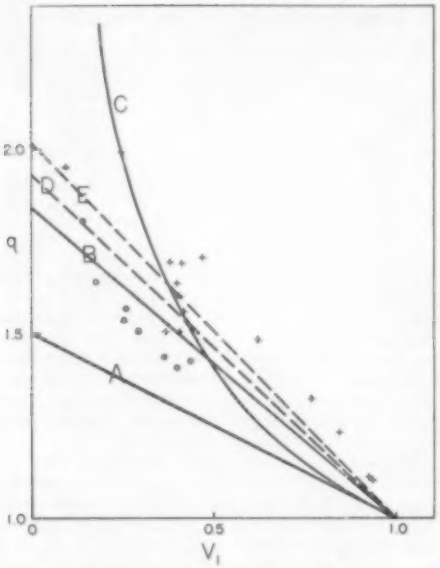

Fig. 3.—Plot of the tortuosity, q, against V_1. The curves A, B, C, D and E refer to theory (see text). ⊙ Are experimental values for random spheres (uniform or a combination of large and small spheres). + Are experiments with random particles (sand, glass powder, carborundum powder, kaolin, soil, etc.).

those for spheres. This is in accord with Eq. 13. Much of the data for random nonspherical particles can be explained by use of Eq. 13 and a $\mathbb{Z}$ value not too different from unity.

Bruggeman's formula (Eq. 14) for spheres predicts too large q at low V_1 (or large V_2) for these data. At large V_1, however, all formulas predict nearly the same q. It is impossible to generalize on the merits of the different formulas on the basis of these data alone. More systematic experimental work on diffusion is desirable over a range of P_2 and P_1 in order to establish the general applicability of any of these formulas.

It is evident that the uncertainty in calculating P_m with any formula for an arbitrary system of nonspherical particles immersed in a continuous medium is quite large. Examination of Eq. 13 reveals that shape effects are not too important percentagewise in the calculation of P_m when P_2/P_1 is near unity. When, however, $P_2/P_1 \gg 1$ or when $P_2/P_1 \ll 1$, shape effects appreciably influence P_m. Particularly when $P_2/P_1 \to \infty$, P_m becomes very sensitive to the particle shape. Then random uniform spheroids with $\mathbb{Z} = 2.0$ or 0.5 may easily give rise to P_m values which may be twice as great as those for uniform spheres with the same V_2.

The choice of $K = 0.78$ employed with Eq. 8 was based on (5) Pearce's selected dielectric data (12) which were mostly in the range of $0.80 > V_2 > 0.2$. Since it is well known that Eq. 8 with $K = 0$ is a good approximation as $V_2 \to 0$, a K value much smaller than 0.78 would perhaps give better agreement at $V_2 < 0.1$. Particularly for emulsions at low concentrations, in which the shape of the internal phase droplet is spherical and particle-particle contacts are few, a K much smaller than 0.78 may be more appropriate. This suggests the use of $K = 0.78$ as an upper limit for a suspension of spheres at $V_2 < 0.1$. Actually at small V_2 values, P_m is generally not influenced too greatly by the choice of K anyway. Therefore $K = 0.78$ used with Eq. 8 appears to provide a useful formula over all V_2.

NONSTATIONARY STATE DIFFUSION THROUGH HETEROGENEOUS BARRIERS

General Concepts.—In the preceding discussions, the attention was directed only to diffusional processes occurring under quasi-equilibrium conditions such that the amount of the diffusing substance necessary to change the concentration in the barrier are of greater importance than the rate of penetration subsequent to the initial breakthrough. Thus the time it takes for water to penetrate through our shoes is of greater importance than the rate of penetration after the barriers have been wetted through. Although in most instances barriers which permit more rapid permeation under steady state conditions also allow more rapid permeation under nonsteady state situations, this is not always true. In this section the time lag for true diffusion in heterogeneous systems is discussed.

Taking the general problem as that illustrated in Fig. 1 one may write for a one-dimensional homogeneous barrier obeying Fick's law and Henry's law.

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \qquad \text{(Eq. 15)}$$

where C is the concentration of the diffusing agent with a diffusion coefficient D in the system, x is the coordinate, and t is time. The particular problem boundary conditions are: $C = C_o$ at $x < 0$ and all t; $C \sim 0$ at $x > h$ and all t; and $C = 0$ at $x > 0$ and at $t = 0$, and it is assumed that equilibrium exists at $x = 0$ between the barrier material and the outside phase.

The solution for this homogeneous case yields (13, 14) for the amount of material, M, penetrating the barrier per unit area per unit time

$$M = \frac{K_a D C_o t}{h} + \frac{2hK_aC_o}{\pi^2}\sum_{n=1}^{\infty}\frac{\cos n\pi}{n^2}\left[1 - \exp\left(\frac{-Dn^2\pi^2 t}{h^2}\right)\right] \qquad \text{(Eq. 16)}$$

Here K_a is the equilibrium partition coefficient of the agent in the two media defined as

$$K_a = \frac{\text{concentration of agent in barrier phase } (0 < x < h)}{\text{concentration in the outside phase } (x < 0)}$$

In Fig. 4 is shown a plot of M/K_aC_o against Dt when $h = 0.1$ cm. When Dt is sufficiently large the system has attained steady state. The intercept of the extrapolation of the steady state (linear) portion of the curve gives the well known lag time of Barrer (14)

$$t = \lambda = h^2/6D \qquad \text{(Eq. 17)}$$

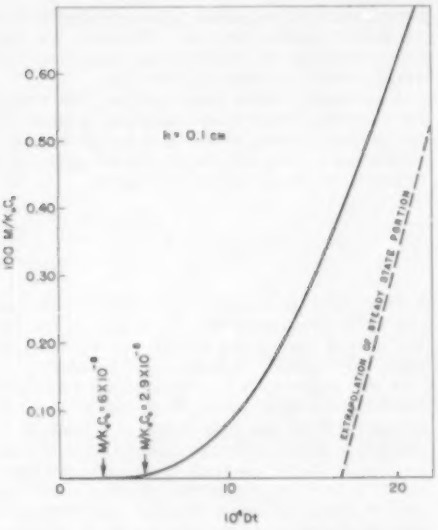

Fig. 4.—Barrier penetration with time.

which is a measure of the period required for the absorption of the agent by the barrier concomitant with the diffusion of the agent through the barrier. In protective film design this quantity is obviously important since it is roughly the measure of the time lapse necessary before the noxious agent begins to make its presence at $x > h$.

It would be desirable to be able to use equations similar to Eqs. 16 and 17 for heterogeneous barriers. It appears that a proper choice of an effective diffusion coefficient, D_m, and an effective partition coefficient, K_m for the polyphase material should be sufficient to give an expression corresponding to Eq. 16.

Emulsion and Suspension Barriers—All Phases Obeying Fick's and Henry's Laws.—Consider the two phase problem in which the time dependent behavior of the agent in the barrier is completely defined by the diffusion coefficients, the partition coefficients, and the volume fractions corresponding to the two phases. A rigorous solution analogous to Eq. 16 appears to be impossible to obtain, but valid approximate solutions under certain conditions are possible.

At sufficiently large times, i. e., in the steady state, the effective permeability constant, P_m, may be given by an appropriate steady state mixture formula, e. g., Eqs. 8, 13, or 14; and the effective partition coefficient is given by $K_m = K_1V_1 + K_2V_2$. The effective diffusion coefficient for steady state is then

$$D_m = P_m/K_m$$

It is now important to establish the criterion for the approximate validity of these effective steady state quantities in nonstationary state problems. Essentially, the cause of the difference between the effective steady state diffusion coefficient and the effective nonstationary state diffusion coefficient is that the average paths for the diffusing molecules may be different in the two cases. However if it can be shown that within a microscopic region of heterogeneity the nonstationary state diffusion picture is essentially that of the steady state, the effective permeability constants should be essentially the same in the two cases. This would be true if the relaxation time, i. e., the time for steady state attainment in the microscopic heterogeneous region is small compared to the time required for a unit change in concentration or activity of the agent in that region.

The smallest heterogeneous volume is that which would just include one particle of the internal phase and the corresponding volume of the external phase. If the rate determining step in the relaxation process is the diffusion into the internal phase particle, then the microscopic relaxation time is approximately (15)

$$\tau \sim \bar{d}^2/15D_2 \qquad \text{(Eq. 18)}$$

where $\bar{d}$ is the equivalent sphere radius of the particle. If the rate determining step is diffusion in the external phase, then the order of magnitude of τ is

$$\tau \sim \frac{2K_m\bar{d}^2}{K_1D_1}\left(\frac{V_1}{V_2}+1\right)^{2/3} \qquad \text{(Eq. 19)}$$

The larger of Eqs. 18 or 19 would be a measure of the microscopic relaxation time in the heterogeneous medium. The smaller this is, the more "homogeneous" the medium appears with respect to diffusion, i. e., the more valid is the concept of the effective diffusion coefficient for use in nonstationary state problems.

The quantity τ must now be compared with $(\Delta t/\Delta C)_x$, the time required for a unit change in concentration. For this purpose it is convenient to use the solution to the one dimensional barrier problem (14) when the dimension h is infinite. In the early part of the diffusion through a membrane of finite thickness, the situation is well approximated by the solution for the infinite case. Furthermore, since it is clear that for any value of x, $\partial C/\partial t$ will be greatest during the early stages of diffusion, it suffices to compare the relative magnitudes of τ and $(\Delta t/\Delta C)_x$ for the infinite barrier problem at small values of t.

The solution to the infinite barrier problem (14) is

$$C = K_mC_0\left[1 - \frac{2}{\pi^{1/2}}\int_o^{\frac{x}{2}(D_mt)^{1/2}} \exp\,(-y^2)dy\right]$$

for which

$$\frac{\partial C}{\partial t} = \frac{K_mC_0X}{2(\pi D_m)^{1/2}t^{3/2}}\exp\left(\frac{-x^2}{4D_mt}\right)$$

The criterion for the applicability of the steady state permeability constant to nonstationary problems is then that

$$\tau \ll \frac{1}{\partial C/\partial t}$$

or that the ratio

$$R = \frac{2(\pi D_m)^{1/2}t^{3/2}}{\tau K_mC_0x}\exp\left(\frac{x^2}{4D_mt}\right) \gg 1 \qquad \text{(Eq. 20)}$$

for the range $0 < x < h$ and $t > 0$.

If Eq. 20 is fulfilled for a suspension or an emulsion system, a curve identical to that in Fig. 4 should be obtained unless Henry's law or Fick's law are not

obeyed for the individual phases. If Eq. 20 is not fulfilled, the M/K_mC_o values for the barrier will always be greater than those given in Fig. 4, hence the barrier would be less effective as a protectant. In this case it would be extremely difficult to obtain an expression for M since the problem involves essentially an effective permeability constant which is both time and concentration dependent.

A close examination of Eqs. 18, 19, and 20 will show that particle size for the internal phase usually limits the fulfillment of Eq. 20 only for systems for which $D_2 \ll D_1$ and is of practical importance when $D_2 \ll D_1$ and $K_2 \gtrsim K_1$, in which case the internal phase particle may not be able to absorb rapidly enough the agent which will be diffusing predominantly in the external phase.

In Fig. 5 are given plots of M against time for barriers whose internal phases are spheres or near spheres with $h = 0.1$ cm., $C_o = 10^{-5}$ moles cm.$^{-3} \sim$ 1 atmosphere, $K_1 = 10^{-2}$, and $D_1 = 10^{-6}$ cm.^{2} sec.$^{-1}$. In these calculations with Eq. 16 it is assumed that Eq. 20 is obeyed and that Eq. 8 with $K = 0.78$ is applicable. Curve A is the case where $V_2 = 0$, i. e., the homogeneous barrier; curve B is $V_2 = 0.3$, $D_2 = 0$, and $K_2 = 0$, i. e., a nonabsorbing suspension; curve C is $V_2 = 0.3$, $K_2 = 10^2K_1$, $D_2 = D_1$, i. e., an absorbing emulsion; and curve D is $V_2 = 0.3$, $K_2 = 10^2K_1$, and $D_2 = 10^{-2}D_1$; i. e., an absorbing solid or semisolid suspension.

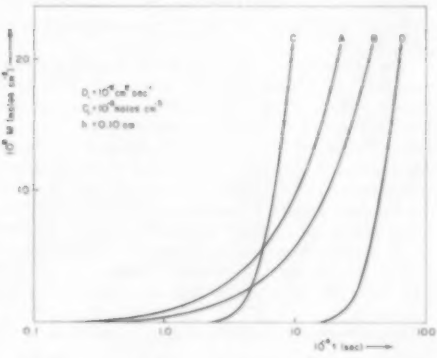

Fig. 5.—Penetration, M, of various heterogeneous barriers (see text) as a function of time.

In terms of the effectiveness of protection case D is by far the best over all t, and case C is superior to cases A and B at low t values but is less effective for very large times. Case B is always somewhat better than case A. In terms of quantitative applicability curve C is the most approximate since, as stated in the section on "Steady State Diffusion," the uncertainty in Eq. 8 is quite large when $P_2/P_1 \to \infty$. Examination of both Bruggeman's formula (Eq. 14) and Eq. 8 with $K = 0.78$ reveals that a maximum in protection, i. e., the maximum in time lag, for the emulsion type barrier with $K_2 \gg K_1$ and $P_2/P_1 \to \infty$ lies in the region of $0.3 > V_2 > 0.2$. Much larger or much smaller V_2 values always lead to smaller lag times and therefore less protection.

It is noteworthy that for small values of t, an emulsion of the type corresponding to curve C may give several orders of magnitude greater protection than an unfilled barrier, curve A. Therefore, for short term protection an emulsion type may most adequately serve the needs of a situation.

A particularly interesting extension of the preceding discussion results if one considers "coating" the particles or droplets of the internal phase with a third phase. Then Eq. 8 may still be applicable if P_2 in the formula is replaced by (16, 17)

$$P_2 = \left[\frac{(2P_o + P_i)(\bar{a} + T)^3 - 2(P_o - P_i)\bar{a}^3}{(2P_o + P_i)(\bar{a} + T)^3 + (P_o - P_i)\bar{a}^3}\right] P_o \qquad \text{(Eq. 21)}$$

where: $P_o = K_oD_o$ is the permeability of the outside phase (coat material), $P_i = K_iD_i$ is the permeability of the inside material, $\bar{a}$ = radius of the inside sphere, and T = thickness of the shell.

In real suspensions or emulsions one may essentially have a third phase "coat" or shell at the boundary of the two main phases as a result of (*a*) adsorption layers of foreign material, (*b*) reaction (oxidation) at the interface resulting in a "skin," or (*C*) a boundary layer with markedly different properties from either the bulk external or the bulk internal phases. In protective barrier formulation there is the possibility of actually "coating" on a third phase layer to decrease the steady state permeability while maintaining the time dependent qualities of the barrier. It can be shown from Eqs. 21, 8, and 16 that a third phase coating of low permeability on emulsion droplets of high K_2 may improve barrier effectiveness, for example, from that given by curve C in Fig. 5 to better than that represented by curve D.

Suspensions and Emulsions Not Obeying Fick's or Henry's Law.—The situation when the individual phases deviate from Fick's law or Henry's law is exceedingly complicated and generally not tractable. There are, however, certain instances of great practical importance with respect to barrier design which may be handled in an approximate fashion.

Consider the system in which the internal phase is composed of particles which may adsorb molecules of the diffusing agent at its surface according to Langmuir's expression (18)

$$m = \frac{k_1k_2C}{1 + k_1C} \qquad \text{(Eq. 22)}$$

where m is the amount of adsorbate per unit mass of adsorbent, C is the equilibrium concentration of the adsorbate, and k_1 and k_2 are the usual constants. If the internal phase material should be characterized by a high k_1, then $m \sim k_2$. Assume that the rate of adsorption is rapid compared to volume diffusion, i. e., the condition analogous to Eq. 20 is fulfilled for this situation.

For such a system, the concentration gradient of the penetrating agent would approximate that shown in Fig. 6. For the idealized case where essentially all of the agent entering into the system is found in the filler phase, the gradient will be strictly linear.

It is evident that the total amount of material which has entered such a barrier is the sum of the material in the external phase and that taken up by the filler. Since the average concentration of the free penetrant up to the point of maximum penetration is $(1/2)K_1V_1C_o$, where C_o is the concentration

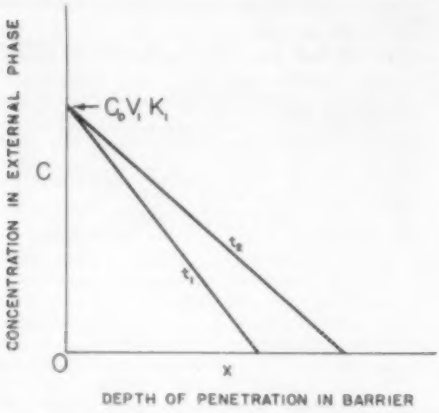

Fig. 6.—Concentration profiles in an ideal adsorption filler barrier at a time t_1 and at a subsequent time t_2.

of the penetrant in the stirred solution (Fig. 1 with $C_h = 0$) in equilibrium with the surface layer of the barrier. The total amount in the external phase is $(1/2)K_1V_1C_ox$, where x is the depth of penetration.

The amount taken up by the filler up to this point is Ax where A is the amount of penetrant adsorbed by the filler per unit volume of the barrier material. The amount of penetrant, dW, necessary to effect an additional penetration, dx, would then be

$$dW = (1/2)K_1V_1C_o dx + A\,dx$$

But,

$$\frac{dW}{dt} = -D_m\frac{dC}{dx} = D_m\frac{K_1V_1C_o}{x}$$

Therefore

$$\frac{D_m K_1V_1C_o}{x} = (1/2)K_1V_1C_o\frac{dx}{dt} + A\frac{dx}{dt}$$

which in integral form is

$$V_1K_1D_mC_o\int_o^\lambda dt = \int_o^\lambda x\,dx[(1/2)K_1V_1C_o + A]$$

Therefore the lag time is

$$\lambda = \frac{h^2}{4D_m} + \frac{h^2A}{2D_mK_1V_1C_o} \qquad \text{(Eq. 23a)}$$

or

$$\lambda = \frac{h^2K_1V_1}{4P_m} + \frac{h^2A}{2P_mC_o} \qquad \text{(Eq. 23b)}$$

P_m is given by Eq. 8 with $P_2 = 0$ for spherical particles or by Eq. 13 for spheroidal particles. The quantity A may be replaced by $A = C_f/k_2$, where C_f is the concentration of the filler (internal) phase and k_2 is one of the constants in Langmuir's Eq. 22.

In the extreme case where none of the penetrant is adsorbed the assumed linear gradient will, of course, not hold; yet it is of some interest to note that even in this instance Eq. 23 predicts a lag time value of $h^2/4D_m$ which is not too markedly different from the true theoretical value of $h^2/6D_m$ from Eq. 17.

Equation 23, it is apparent, also applies to situations where the filler phase reacts chemically to immobilize or destroy the penetrating agent. This is a very common situation whose behavior the derived relationship will predict quite accurately, particularly if the reacting component is uniformly distributed either as fine particles or as solute. Indeed the same equation in a slightly different form can predict the rate of release of dispersed drug crystals, for example from ointment bases. It evidently has broad general applicability.

GLOSSARY OF SYMBOLS

A	= amount of agent taken up by filler per unit volume of barrier material
$A\bar{a}$, $A\bar{b}$, $A\bar{c}$	See Eq. 10
a	= activity of agent
a_o, a_h	= activities of agent in solutions to the left of barrier and to the right of barrier, respectively
$\bar{a}$	= radius of sphere or a semiaxis of an ellipsoid
$\bar{b}$, $\bar{c}$	= other semiaxes of an ellipsoid
C	= concentration of agent
C_o, C_h	= concentrations of agent in solutions to the left and right of barrier, respectively
C_f	= concentration of filler in barrier
D	= diffusion constant of agent
D_1, D_2	= diffusion constants of agent in phases 1 and 2, respectively
D_m	= effective diffusion constant of agent in heterogeneous barrier
D_i, D_o	= diffusion constants, see Eq. 21
F_1, F_2	= activity gradients of agent in phases 1 and 2, respectively
F_o	= average activity gradient of agent in heterogeneous barrier
F_1', ΔF_1	= see Eq. 6
h	= thickness of barrier
K	= A coefficient in Eq. 8
K_d	= partition coefficient of agent, see Eq. 1a
K_1, K_2	= partition coefficients of agent in the solution to the left of barrier and phases 1 and 2, respectively
K_m	= effective partition coefficient of agent in the heterogeneous phase and the solution to the left of barrier
K_i, K_o	= see Eq. 21
k_1, k_2	= see Eq. 22
L	= see Eq. 13
M	= amount of agent having penetrated through barrier
m	= see Eq. 22
n	= an integer
p	= rate of permeation
P	= permeability constant of system for agent
P_1, P_2	= permeability constants for agent in phases 1 and 2, respectively (referred to solution left of barrier)
P_o, P_h	= permeability constants for the agent in barrier referred to the solutions to the left and the right of barrier, respectively
P_i, P_o	= permeability constants, see Eq. 21
P_m	= effective permeability constant of agent in heterogeneous barrier referred to solution to the left of barrier
q	= tortuosity
r	= radial coordinate
s	= a variable
t	= time
T	= thickness of coat
V_1, V_2	= volume fractions of phases 1 and 2, respectively
W	= amount of agent adsorbed by barrier
x	= a coordinate
$\mathfrak{A}$	= axial ratio for spheroid
γ	= activity coefficient of agent
γ_1, γ_2	= activity coefficients for agent in phases 1 and 2, respectively
γ_o, γ_h	= activity coefficients for the agent in solutions to the left and right of the barrier, respectively
γ_m	= effective activity coefficient of agent in heterogeneous barrier
λ	= lag time
τ	= relaxation time
ϕ	= perturbation potential

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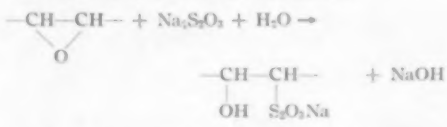
Determination of Basic α-Epoxides*

By J. B. LEARY

A common disadvantage of methods for determining basic epoxides or epoxides in the presence of a base is the necessity for determining a blank correction. A fast, direct method based on reaction with a standard solution of sodium thiosulfate is presented. The excess sodium thiosulfate is titrated with standard iodine solution. No blank correction is necessary. The stoichiometry of the reaction, the effect of temperature on the rate of reaction, precision, and other factors are described.

THE USE of certain epoxides as tumor inhibitors was first reported by Hendry, *et al.* (1, 2). The therapeutic activity of basic bis-epoxides in murine leukemia and other transplantable mouse neoplasms has been demonstrated by Johnson and Wright (3). The effects of epoxypropyl piperazine derivatives on transplanted mouse leukemia spectra have been described (4). Clinical studies of 1,4-bis(2,3-epoxypropyl)piperazine and other poly-functional alkylating agents has been reported (5). Gerzon, *et al.*, have studied the relationship between structure and antileukemic activity of some diamine bis-epoxides (6). These studies resulted in the necessity for an accurate and reproducible method for determining basic epoxides.

All of the epoxides studies were water soluble and were to be administered parenterally. This necessitated the use of a method of determination which could be used on dilute aqueous solutions. Jungnickel, *et al.* (7), have thoroughly reviewed the many methods for determining the α-epoxy group and have set forth a list of recommended methods after consideration of the physical characteristics of the epoxide to be determined and the physical form of the sample. The recommendations for dilute aqueous solutions of α-epoxides include the method by Ross (8) based on esterification by thiosulfate as follows:



The method tends to be tedious since continuous titration of the liberated base with acetic acid is required to prevent hydrolysis of the thiosulfate derivative. Results obtained by this method in this laboratory on certain epoxides tended to be inaccurate (low) and were difficult to reproduce. A similar method based on the reaction between α-epoxides and sodium sulfite with subsequent liberation and titration of sodium hydroxide with hydrochloric acid was reported by Kireev (9) and Swan (10) for determining various other α-epoxides. A common disadvantage of the above mentioned methods is the necessity for determining a blank correction for basic epoxy compounds. Ross has mentioned that satisfactory results were obtained on more reactive compounds by carrying out the thio-

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sulfate reaction in the presence of excess acetic acid. In the case of less reactive compounds, prolonged heating resulted in the decomposition of thiosulfate and deposition of sulfur. A rapid and precise method based on reaction with thiosulfate in the presence of excess acetic acid followed by titration of excess thiosulfate with iodine is herein described.

EXPERIMENTAL

Temperature.—Although the Ross method required reflux temperature conditions, the rate of reaction at lower temperatures was not reported. Since lower temperatures would be more convenient, an investigation of temperature conditions was carried out. The effect of temperature on the rate of reaction of sodium thiosulfate with N,N′-bis(2,3-epoxypropyl)piperazine is shown in Table I.

TABLE I.—THE EFFECT OF TEMPERATURE ON THE RATE OF REACTION OF SODIUM THIOSULFATE WITH N,N′-BIS(2,3-EPOXYPROPYL)PIPERAZINE

Temperature, °C.	Time of Reaction	Epoxide Consumed, %
25	5 min.	55.3
	55 min.	88.8
	110 min.	93.8
	18 hr.	96.5
70	5 min.	95.7
	10 min.	98.6
	15 min.	99.4

Thus it is shown that at 70° complete reaction is obtained in fifteen minutes. All of the epoxides studied proved to react completely under these conditions.

Stoichiometry.—Under certain conditions the reaction between epoxides and sodium thiosulfate is quantitative, but little is known about the stoichiometry of the reaction. To answer this question an experiment was designed to determine the required excess thiosulfate to obtain quantitative results. Holding the epoxide sample size constant, various amounts of thiosulfate were added.

TABLE II.—EFFECT OF EXCESS THIOSULFATE ON EPOXIDE REACTION

Meq. $Na_2S_2O_3$/Meq. Epoxide	Epoxide, %
1.2	93.8
1.5	95.3
1.8	96.8
2.0	97.8
2.2	98.0
2.5	98.0

The results, shown in Table II, indicate the necessity of at least a onefold excess of thiosulfate over epoxide. In other words, for every milliequivalent of epoxide in a sample, two milliequivalents of sodium thiosulfate should be added for complete reaction and quantitative results.

Acetic Acid Excess.—Another factor to be considered was the amount of acetic acid necessary in the reaction mixture. Theoretically equivalent amounts of acetic acid and epoxide are necessary to prevent hydrolysis of the epoxide by the sodium hydroxide produced by the primary reaction. However, the possibility remains that the reaction would not go to completion unless an excess of acetic acid was present. Holding the sample weight and sodium thiosulfate volume constant, various amounts of acetic acid were added. The results shown in Table III indicate the necessity of a 25% excess of acetic acid in the reaction mixture.

TABLE III.—EXCESS OF ACETIC ACID IN EPOXIDE REACTION

Meq. Acetic Acid/Meq. Epoxide	Epoxide, %
0.94	91.2
1.00	95.3
1.06	96.3
1.20	98.3
1.25	99.4
1.40	99.4

Table IV lists the results obtained on different basic epoxides prepared at the Lilly Research Laboratories by Gerzon, *et al.* (6).

It was noted that the purity of different lots of some of these compounds was consistently high, whereas the purity of different lots of other compounds was consistently low (see Table V). To help explain whether this phenomenon is due to a discrepancy in the method of determination or to the presence of polymeric material the conditions for optimum results were reinvestigated for compound VII. The results indicated that optimum conditions were the same for compounds I and VII.

Reproducibility.—Ten replicate determinations were made on a sample of compound I, and the standard deviation was found to be 1.3%.

Reagents.—*Iodine.*—A 0.1 *N* solution is prepared and standardized by the recommended U. S. P. XV method. *Sodium thiosulfate.*—A 0.1 *N* solution is prepared and standardized *versus* standard iodine solution before each series of determinations. *Starch* T.S. *Acetic acid.*—A 0.250 *N* solution is prepared by diluting 7.1 ml. of glacial acetic acid to 500 ml. with distilled water and adjusting the normality to exactly 0.250.

METHOD

Transfer an accurately weighed sample or aliquot of an aqueous solution equivalent to approximately 1.0 meq. of epoxide to a 50-ml. Erlenmeyer flask containing 5.0 ml. of 0.25 *N* acetic acid. Add 25.0 ml. of standard 0.1 *N* sodium thiosulfate. After mixing, place the flask in a 70° temperature bath for fifteen minutes. Cool the solution quickly in an ice bath and immediately titrate with standard 0.1 *N* iodine using starch T. S. as an indicator. Calculate the amount of sodium thiosulfate consumed, and from this determine the amount of epoxide in the sample.

TABLE IV.—RESULTS OBTAINED ON VARIOUS BASIC EPOXIDES

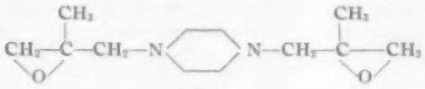
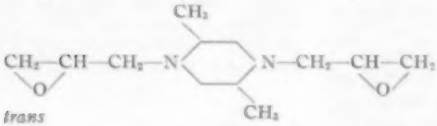
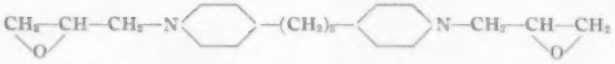
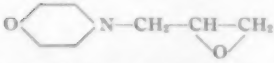
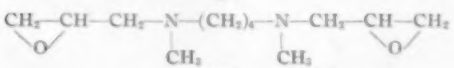
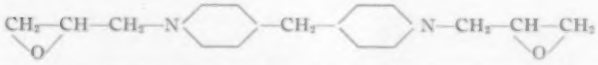
I		99.8%
II		100%
III		100%
IV		89.0%
V		99.5%
VI		96.5%
VII		91.0%
VIII		85.0%

TABLE V.—RANGE OF RESULTS ON VARIOUS LOTS OF COMPOUNDS I AND VII

Compound I		Compound VII	
Lot	Epoxide, %	Lot	Epoxide, %
1	100	1	91.2
2	100	2	91.0
3	99.9	3	89.7
4	99.8	4	89.5
5	99.4	5	89.1
6	98.0	6	89.0
7	98.0	7	88.0
8	97.8	8	87.9
9	96.5	9	87.6
10	96.4	10	87.5

SUMMARY

A method for determination of basic epoxides has been described which is based on reaction with thiosulfate and iodimetric measurement of the excess thiosulfate. Experimental conditions for quantitative results have been investigated. The method has been found to be accurate and reproducible.

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The Action of Ethyl Carbamate on Oxidative Phosphorylation*

By KWAN-HUA LEE

Ethyl carbamate inhibits α-ketoglutarate oxidation and phosphorylation proportionately, but does not affect succinate oxidation or its coupled phosphorylation in rat liver mitochondrial preparations. Oxidative phosphorylation uncoupling effect is not a general mechanism of action of narcotics.

THEORIES have been proposed for the mode of action of narcotics including general anesthetics, hypnotics, and analgesics, yet none is universally accepted (1). The lack of a pronounced inhibition of oxygen consumption by the barbiturates in therapeutic concentrations suggested the possibility that these drugs may exert their effect by an uncoupling of oxidative phosphorylation. Brody and Bain (2) found that most of the few barbiturates they studied showed inhibition of both respiration and phosphate uptake in rat brain and liver mitochondrial preparations. The inhibition of phosphate uptake was greater than the inhibition of respiration and thus the P/O was decreased as drug concentrations were increased. However, their results were not in agreement with those obtained by Eiler and McEwen (3) in an earlier study on the effect of pentobarbital on oxidative phosphorylation in a rat-brain homogenate preparation. Later, Brody and Bain (4) explained that the "phosphorylation uncoupling effect" of barbiturates can only be demonstrated in isotonic sucrose mitochondrial preparations but not in water or saline homogenate preparations. Recently, Low, *et al.* (5), reported that amobarbital at a concentration of 1.8×10^{-3} *M* blocks completely those mitochondrial oxidations which proceed via pyridine nucleotides but do not affect the oxidation of succinate nor the associated phosphate uptake.

In this paper, the results of the studies on the effect of urethane (ethyl carbamate), a representative of another class of hypnotics, on oxidative phosphorylation in rat liver mitochondrial preparations are reported. We selected α-ketoglutarate and succinate as two representative substrates with different oxidative pathways. It was found that when α-ketoglutarate was used as the substrate, urethane inhibited respiration and phosphorylation proportionately even at a higher concentration of drug (0.3 *M*) where respiration was reduced to only one-half of the control. When succinate was used as the substrate, the respiration and phosphate uptake were slightly inhibited at lower concentrations of urethane (0.05–0.1 *M*) and the inhibitions were not further increased as the concentration of urethane was increased. When urethane concentration was greater than 0.3 *M*, there was a sharp fall of P/O regardless of the substrate used. These results are discussed in the text.

METHODS AND MATERIALS

Preparation of Mitochondria.—Ten grams of liver were quickly excised from decapitated albino rats and immediately minced in an ice-chilled glass mortar and then ground into a paste. Four volumes of 0.32 *M* sucrose solution were introduced into the paste with gentle stirring. This suspension was then transferred into a Dounce homogenizer (6), chilled in an ice bath, and homogenized with eight passes of the "loosely fitted" plunger. The homogenate was then diluted with an equal volume of a 0.25 *M* sucrose solution and then centrifuged in a Sorvall refrigerated centrifuge at 700 × g for ten minutes. The supernatant was collected and centrifuged at 13,000 × g for ten minutes. The residue was washed with 20 ml. of 0.25 *M* sucrose solution and then centrifuged at 13,000 × g for another ten minutes. The washing of the residue was repeated once more. The final residue was evenly suspended in 10 ml. of 0.25 *M* sucrose solution and used as mitochondrial preparation. During the decantation of the supernatants, attempts were made to get rid of all of the fluffy substances. All of the above procedures were carried out at ice temperature.

Measurement of Oxidative Phosphorylation.—Double-armed Warburg vessels in duplicates chilled in an ice bath were charged with ice cold reaction mixture and the urethane solution of various concentrations in the main compartment. In one of the side arms was added 0.3 ml. of a 70% trichloroacetic acid solution and to the other 0.5 ml. of hexokinase in glucose solution. A small roll of filter paper was placed in the center well containing 0.2 ml. of 20% KOH solution. Mitochondrial preparation was the last one added to the main compartment. After a ten-minute period of temperature equilibrium at 27°, trichloroacetic acid was tipped in the blank control flasks and hexokinase was tipped in the rest of the flasks. Oxygen uptake was measured for twenty minutes. The reaction was

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stopped by tipping in the trichloroacetic acid in the side arm. Five milliliters more of 7% trichloroacetic acid solution was added to each flask and the mixture was centrifuged. The inorganic phosphate content in the clear extract was determined according to Fiske and Subbarow (7).

Hexokinase used in this experiment was prepared from yeast and purified to the 3a stage as described by Berger, *et al.* (8). Crystalline ATP sodium salt was purchased from California Foundation for Biochemical Research. Ethyl carbamate was a Merck U. S. P. preparation which had been recrystallized three times. α-Ketoglutaric acid was a product of Nutritional Biochemicals Corporation, and was recrystallized twice from ethyl propionate in order to remove the trace amount of succinic acid usually present according to Price (9). Other chemicals used were Merck C. P. grade preparations. All of the solutions were adjusted to pH 7.4 if necessary.

RESULTS AND DISCUSSION

The effect of urethane on respiration and phosphate uptake in rat liver mitochondrial preparation with α-ketoglutarate or succinate as the substrate is presented in Tables I and II, respectively. The data are also plotted as per cent of control in Figs. 1 and 2. Data in Table I definitely indicate that, when α-ketoglutarate was used as the substrate, urethane inhibited both the respiration and inorganic phosphate uptake proportionately and P/O values remained essentially constant throughout a wide range of drug concentrations. When the concentration of urethane reached 0.3 *M*, the respiration was reduced to only one-half of the control level and the P/O was still as high as the control. When succinate was used as the substrate (see Table II and Fig. 2) there was a slight decrease in P/O at lower concentrations of urethane (0.05–0.1 *M*), but the P/O was not further decreased as the concentrations of urethane were increased. In both cases, when the concentration of urethane was higher than 0.3 *M*, there was a sharp drop of P/O. At a very high concentration of urethane (0.5 *M*), there was no change in phosphate concentration as compared with the blank control. The effect of urethane on respiration as studied with α-ketoglutarate or succinate as substrate supports the classical work of Jowett and Quastel (10) and the later work of Greig (11) that the action of narcotics on respiration is located on the electron path between mitochondria DPN linked dehydrogenases and the common point in the respiratory chain, where the electron transport from these dehydrogenases and from succinate enter the cytochrome system, commonly referred to as Slater's factor.

TABLE I.—EFFECT OF URETHANE ON α-KETOGLUTARATE OXIDATION AND PHOSPHORYLATION[a]

Concentration of Urethane, *M*	Oxygen Uptake, μ Atom	Phosphate Uptake, μ Mole	P/O
Control	7.76	28.2	3.65
0.05	7.73	27.2	3.51
0.20	6.02	21.4	3.57
0.30	4.36	15.5	3.58
0.40	2.52	7.15	2.84
0.50	0.77	0.0	...

[a] Each Warburg vessel contained the following substances in micromoles: ATP 6, IP 38.5, Cyt. C 3.6×10^{-2}, $MgSO_4$ 23, KF 30, α-ketoglutarate 30, malonate 15, urethane 0–1500, rat liver mitochondria 0.5 ml. The final volume was 3.0 ml., including 0.1 ml. hexokinase and 9 mg. glucose in 0.4 ml. 0.25 *M* sucrose solution.

TABLE II.—EFFECT OF URETHANE ON SUCCINATE OXIDATION AND PHOSPHORYLATION[a]

Concentration of Urethane, *M*	Oxygen Uptake, μ Atom	Phosphate Uptake, μ Mole	P/O
Control	10.3	19.3	1.87
0.05	9.92	17.2	1.73
0.1	9.0	15.0	1.67
0.2	9.25	15.6	1.70
0.3	9.20	15.6	1.70
0.4	8.90	8.55	0.96
0.5	7.12	0.0	...

[a] The reaction mixture was essentially the same as described in Table I except 30 micromoles of Na-succinate was used instead of α-ketoglutarate and malonate.

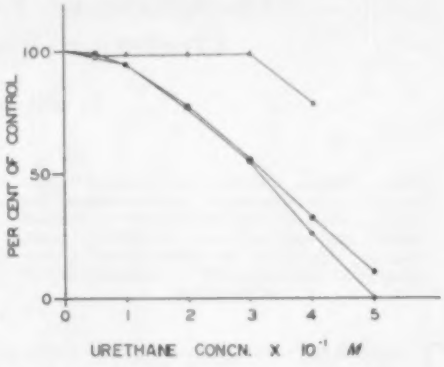

Fig. 1.—Effect of urethane on α-ketoglutarate oxidation and phosphorylation. ▲——▲ P/O, O——O oxygen uptake, ●——● inorganic phosphate uptake.

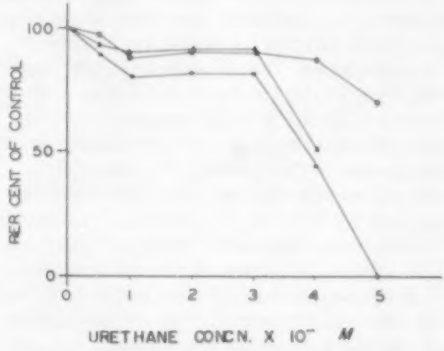

Fig. 2.—Effect of urethane on succinate oxidation and phosphorylation. ▲——▲ P/O, O——O oxygen uptake, ●——● inorganic phosphate uptake.

This was shown by the fact that urethane inhibited respiration in rat liver mitochondrial preparation when α-ketoglutarate was used as the substrate but not when succinate was used.

The present results definitely deny that an "uncoupling effect" was involved in the action of urethane or as the mode of action of narcotics in general. It is not justified to consider the effect of urethane at very high concentration as drug action. It is well known that urethane is a powerful denaturing agent. The denaturation effect of urethane at high concentrations on proteins or enzymes is a general phenomenon (12).

The inhibiting effect of very high concentration of urethane on oxidative phosphorylation observed in the present study was probably due to the denaturation action of the drug on enzyme or enzymes involved in the reaction mixture.

SUMMARY

The effects of urethane on the oxygen uptake and phosphate uptake in rat-liver mitochondrial preparation using α-ketoglutarate or succinate as substrate have been studied. Urethane inhibited α-ketoglutarate oxidation but not succinate oxidation and it did not show uncoupling effect in either case. When urethane concentration was higher than 0.3 *M*, there was a sharp fall of oxygen uptake and P/O; this was probably due to a general denaturation of the enzyme system.

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The Synthesis of β-Aminoethyl Ketones as Potential Antagonists of β-Alanine*

By SHU-SING CHENG† and SIGURDUR JONSSON

The organocadmium reagent was adapted to the synthesis of alkyl β-aminoethyl ketones from β-alanine. Some aryl β-aminoethyl ketones were synthesized from β-alanine through the Friedel-Crafts reaction. These two series of β-aminoethyl ketones were proposed as potential antagonists of β-alanine in the metabolism of certain microorganisms. Both the alkyl and aryl β-aminoethyl ketones show antibacterial effect when tested against *S. aureus* and *E. coli in vitro*.

THIS REPORT deals with analogs of the amino acid β-alanine, designed as potential antagonists of possible biochemical or chemotherapeutic interest. This amino acid is significant in biological metabolism, as shown by its presence in the molecule of pantothenic acid, which is an essential component of coenzyme A (1).

In the search for metabolic antagonists of pantothenic acid, much work has been done on the synthesis of structural analogs with pantoic or β-alanine moiety modifications (2, 3). These structural analogs were designed and synthesized in the hope that they might interfere with the metabolism of those microorganisms which require preformed pantothenate. Analogs of β-alanine itself might be of interest as possible inhibitors of those microorganisms which synthesize pantothenic acid themselves from β-alanine and pantoic acid. β-Alanine has been shown to be a growth factor for various microorganisms (4–9), but not much work has been done in synthesizing compounds that might act as antagonists or displacers thereof (10).

Numerous instances of antagonism between metabolite and antimetabolite have been observed by substituting various isosteric groups for a carboxyl group of a metabolite (11). Among these is the keto fragment —COR or —COAr (12, 13). It seemed, therefore, of interest to prepare ketone analogs, $H_2NCH_2CH_2COR$

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From a thesis submitted by Shu-Sing Cheng to the Graduate School of the University of North Carolina in partial fulfillment of the requirements for the degree of Master of Science.
† Recipient of the Lunsford Richardson Pharmacy Award.

or $H_2NCH_2CH_2COAr$, of β-alanine as possible antagonists to β-alanine itself. The amino group may be left intact in the hope that it can still couple with pantoic acid to give pantothenic acid analogs with a keto fragment in place of the carboxyl group. The analogs thus formed would not be able to couple with cysteine with the result that the biosynthesis of coenzyme A in the microorganism might be inhibited. A possible mode of action similar to this has been visualized by Mika and co-workers (14) while studying the growth-inhibiting effect of α-alanine on *Brucella*. Alternately, the compound might react with the enzyme involved in the coupling of pantoic acid with β-alanine and render it inactive.

A survey of the literature for the synthesis of amino ketones showed that none of the published methods was suitable for the general synthesis of ketones of the formula $H_2NCH_2CH_2COR$, where R is an alkyl group. The organocadmium reagent has been widely used for the synthesis of polyfunctional keto compounds since its introduction by Gilman and Nelson in 1936 (15); however, it seems never to have been used for the preparation of amino ketones. The synthesis of the β-aminoethyl alkyl ketones reported here was accomplished by the reaction of β-phthalimidopropionyl chloride and the proper dialkylcadmium and subsequent hydrolysis of the phthaloyl group.

The β-aminoethyl aryl ketones, $H_2NCH_2CH_2COAr$, were synthesized by the Friedel-Crafts ketone synthesis from β-phthalimidopropionyl chloride and benzene, or substituted benzene, and subsequent hydrolysis of the phthaloyl group. These ketones were synthesized so as to test the possibility of enhanced biological activity of the aryl radical adjacent to the carbonyl group (16).

EXPERIMENTAL

All melting points were taken with the Fisher-Johns apparatus.

β-Phthalimidopropionic acid was prepared according to Gabriel (17). Amide, flat needles from water, m. p. 204–205°; anilide, needles from water, m. p. 189°.

β-Phthalimidopropionyl chloride was prepared according to Gabriel (18). A better yield (93.5%) was obtained by substituting thionyl chloride for the phosphorus pentachloride.

The β-phthalimidoethyl alkyl ketones prepared are listed in Table I and the alkyl β-aminoethyl ketones hydrochlorides obtained therefrom by hydrolysis in Table II. Representative examples of the preparation follows:

1-Phthalimido-3-heptanone.—The cadmium alkyl was prepared in the conventional way (19, 20) from 54.66 Gm. (0.4 mole) *n*-butyl bromide through the Grignard reagent and replacement of the ether by 80 ml. of anhydrous benzene. The flask was cooled in ice and a solution of 65.4 Gm. of β-phthalimidopropionyl chloride (0.27 mole) in 300 ml. of anhydrous benzene was gradually added over a period of twenty minutes, while the mixture was stirred vigorously. The mixture solidified and the ice-bath was then removed. When heat evolution subsided, the mixture was refluxed for three hours. The mixture was then cooled in an ice bath and acidified with dilute hydrochloric acid. The clear aqueous solution was separated from the benzene solution and extracted with several portions of benzene. The benzene extracts were combined, washed with ether, several portions of a 10% solution of sodium bicarbonate, and again with water, and finally dried over anhydrous sodium sulfate. The benzene was removed *in vacuo* yielding a residue of crude 1-phthalimido-3-heptanone. The crude ketone was purified by recrystallization from alcohol-water.

1-Amino-3-heptanone (n-Butyl β-Aminoethyl Ketone) Hydrochloride.—Three grams of 1-phthalimido-3-heptanone, 10 ml. concentrated HCl, and 15 ml. glacial acetic acid were refluxed for three hours, another 10 ml. concentrated HCl added, and refluxing continued for three more hours. The mixture was concentrated *in vacuo* to remove excess acid and water added until no more precipitate formed. The precipitate was filtered off, washed with water, and the washings combined with the filtrate. The filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in the minimum amount of absolute alcohol, and ether was added dropwise until further addition caused no more precipitation. The precipitate was collected by filtration, washed with ether, and dried.

The β-phthalimidoethyl aryl ketones prepared are listed in Table I and the aryl β-aminoethyl ketone hydrochlorides, obtained therefrom by hydrolysis, in Table II. These ketones were prepared by the conventional Friedel-Crafts acyl condensation from the β-phthalimidopropionyl chloride, anhydrous aluminum chloride, and an excess of the aromatic hydrocarbon. The aryl β-aminoethyl ketone hydrochlorides were obtained by hydrolysis of the phthalimido derivative as described above.

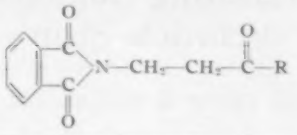
ANTIMICROBIAL EFFECTS

In preliminary tests all of the aminoketones here reported completely prevent the growth of *E. coli* ATCC 8739 in a synthetic medium, the 2,5-dimethylphenyl β-aminoethyl ketone hydrochloride and the *n*-heptyl β-aminoethyl ketone hydrochloride in a concentration of 250 γ/ml.; the others at a concentration of 500 γ/ml. The first two also completely prevent the growth of *Staph. aureus* in nutrient broth at the concentration of 100 γ/ml. and 500 γ/ml., respectively.

DISCUSSION

The low yield of the alkyl β-phthalimidoethyl ketones can be accounted for by the volatility of the dialkylcadmium. Since the replacement of ether

TABLE I.—ALKYL AND ARYL β-PHTHALIMIDOETHYL KETONES



R	M. p., °C.	Yield, %	Analyses[a] Calcd., % C	H	N	Found, % C	H	N	Derivative: M. p. of 2,4-Dinitrophenylhydrazone, °C.
n-Butyl	102.5	20.1[b]	69.28	6.87	5.38	69.13	6.87	5.21	152–153
n-Hexyl	100	14.6[b]	70.88	7.35	4.86	70.76	7.27	4.88	129–130
n-Heptyl	90	16.6[b]	71.55	7.67	4.65	70.75	7.42	5.15	131–132
Phenyl	130–131[c]	71	73.11	4.66	5.02	72.74	4.80	4.61	274–275
p-Methylphenyl	135	78	73.71	5.12	4.78	73.95	5.38	4.84	...
2,5-Dimethylphenyl	136–137	71	74.26	5.54	4.56	74.23	5.76	4.50	...

[a] Analyses by Weiler and Strauss, Oxford, England. [b] Yield of twice recrystallized material. [c] Literature reports 131–132° (18); 130° (21).

TABLE II.—ALKYL AND ARYL β-AMINOETHYL KETONE HYDROCHLORIDES
$H_2NCH_2CH_2COR \cdot HCl$

R	M. p.,[a] °C.	Analyses[a] Calcd., % N	Found, % N	Derivative: M. p. of Picrolonate, °C.
n-Butyl	142[b]	8.40	8.27	179 (decompn.)
n-Hexyl	135[c]	7.23	7.2	175 (decompn.)
n-Heptyl	132	6.74	6.77	172 (decompn.)
Phenyl	126–127[d]	7.54	7.57	185–200[e]
p-Methylphenyl	193–194	7.02	6.76	186–204[e]
2,5-Dimethylphenyl	173	6.56	6.76	176–187[e]

[a] Analyses by Weiler and Strauss, Oxford, England. [b] Literature reports 142–143° (22). [c] Literature reports 162° (22). [d] Literature reports 127° (21). [e] Decomposes slowly in this range without melting.

with benzene is necessary for the condensation of the dialkylcadmium and the acyl chloride, the volatility of the dialkylcadmium is one of the factors limiting the yield.

The synthesis of phenyl and methylphenyl β-phthalimidoethyl ketones by the Friedel-Crafts acylation provides evidence that only one acyl group can be introduced into the aromatic nucleus. This coincides with the general observation that, in the Friedel-Crafts ketone synthesis, the reaction stops abruptly with the introduction of a single acyl group, so that the primary condensation product is not contaminated with products of more than one acyl group. The condensation of β-phthalimidopropionyl chloride with toluene gives methylphenyl β-phthalimidoethyl ketone. The infrared absorption spectrum of this compound shows that it is the *para*-isomer. This fact coincides with the general observation and may be properly explained as a steric effect (23).

The preliminary antibacterial tests do not show if the compounds reported here are true β-alanine antagonists or not. It is hoped this will be investigated at a future date. However, *E. coli*, which grows in a medium free of pantothenic acid, and hence supposedly synthesizes it's own supply, is strongly inhibited by the compounds reported here. On the other hand it is not inhibited by pantoyltaurine (24), a well known pantothenic acid antagonist. This lends support, indirectly, to the suggested mechanism of antibacterial effects of the β-aminoethyl ketones.

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A System for Measuring Contractions of the Rat Ventricle Strip*

By DUANE G. WENZEL, IVENS A. SIEGEL, and ROBERT E. NICHOLS

A system is described for the quantitative measurement of the contractions of the electrically driven rat ventricle strip. The apparatus includes the components of the transducer-amplifier-recording system as well as a simplified muscle-holder and electrode assembly. As normally employed, the system accurately measures semi-isometric (>0.025 mm. displacement) muscle contractions at a stimulus frequency of four per second. It may also be employed at other frequencies and for moderate isotonic contractions as well as for gas displacement.

THE QUANTITATIVE measurement of muscle contraction is the basis of a considerable portion of experimental pharmacology. Systems for measuring such contractions require some device by which the distance that a muscle moves or the force with which it contracts is quantitatively converted into a measurable record. Such devices are termed transducers. Systems in common use are the simple mechanical lever, the optical lever, and the various mechanoelectrical systems in which the muscle displacement is quantitatively converted into an electrical signal, the signal amplified, and, in turn, used to drive an electromechanical or electrooptical recorder. For an excellent discussion of the various transducers employed in biological studies the reader is referred to the work of Machin (1).

The following paper describes a versatile system in which a variable inductance type of mechanoelectrical transducer is employed. While the system to be described was designed specifically for the semi-isometric measurement (>0.025 mm. displacement) of the electrically driven rat ventricular strip (2), almost any driven or spontaneously contracting muscle preparation may be studied with only minor modifications. For example, as the transducer employed uses a bellows as a spring to control the movement of the core, the apparatus may also be used to measure air or gas displacement or, by substituting a light spring for the bellows, it is effective for measuring moderate isotonic contractions.

* Received January 30, 1960, from The University of Kansas, School of Pharmacy, Lawrence.

The investigation leading to the development of this apparatus was supported in part by a research grant (H-3842) from the National Heart Institute, Public Health Service and by a research grant from the University of Kansas.

INSTRUMENTAL

A block diagram of the total transducer system is shown in Fig. 1. All electrical connections between the units of the system are made with shielded cable.

The transducer itself is a model 1JD2578 Westinghouse barometric rate control unit. This is rigidly mounted in and electrically insulated from a 5 × 6 × 4-inch case. The moveable transducer core is pivoted to an arm to which the muscle is attached. This arm projects through an opening cut in the front of the case. The cased transducer is in turn attached to the rack of a Babco No. 945 drill press stand. By substituting an expansion spring for the compression spring of the drill press, the verticle adjustment of the transducer case may be finely controlled with what was originally a depth-limiting screw of the drill press. This makes it possible to adjust the position of the transducer relative to the muscle chamber and to control the tension placed on the muscle.

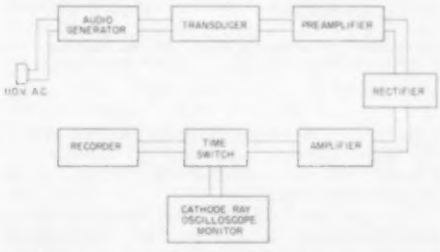

Fig. 1.—Block diagram of the electrical components of the system.

One of the disadvantages of the variable inductance transducer is a susceptibility to vibration, a drawback related to its extreme sensitivity. To minimize this effect, the mass of the apparatus is increased by substituting a solid steel rod 22 inches long by 1¼ inches in diameter for the hollow vertical rod of the drill press, and mounting the apparatus on a base of Colorlith desk top 20 × 15 × 1¼ inches. When this base is placed on several butyl rubber stoppers, the vibrational noise is minimized.

A Heathkit model AG-9A audiogenerator is used to energize the transducer coil. The rectifier operates at an audiogenerator frequency of 20 kc. and a voltage of 0.5 was found to be satisfactory. The signal from the transducer is first amplified by a Tektronix type 122 low level preamplifier with a voltage gain of 100 and a frequency response between 80 cycles and 40 kilocycles. The output of the preamplifier is then rectified in order to remove the 20 kc. carrier wave. A circuit diagram for the rectifier is shown in Fig. 2. The frequency response of this system is essentially flat between one and twenty c.p.s. The d. c. output of the rectifier is

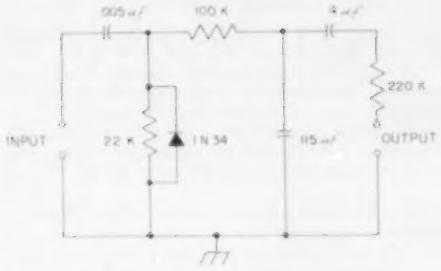

Fig. 2.—Circuit diagram of the rectifier.

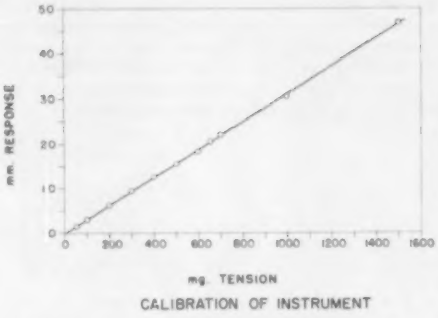

Fig. 3.—The relation between the amount of tension placed on the transducer and the response of the recorder.

further amplified by either an Epsco model 8108-B or a model 8122-A amplifier. In the experiment for which the apparatus was designed, that is to follow a stimulus frequency of four per second, either amplifier is adequate. However, for possible applications in which a relatively steady state is to be measured, the 8122-A amplifier will not provide adequate gain as the d. c. gain of this instrument is only 150. A continuously variable gain control on amplifier controls the final voltage output to an appropriate recording device. For the application to be described, it is desired to automatically record the results for a ten-second period every five minutes. This is accomplished by the use of a recycling cam timer. This timer is used to alternate the signal between a Brush model BL201 oscillograph for ten seconds and a Tektronix type 360 oscilloscope, which was used to monitor the results, for the remainder of the five minutes. For recording at frequencies of less than two per second, a Texas Instruments Recti-Riter provides a rectilinear record of greater amplitude. For studies in which frequencies higher than about 10 per second are to be employed, it is necessary to modify the system of rectification.

The linearity of the system was measured by adding weights to the transducer arm and recording the response on the oscillograph. Response was linear as shown in Fig. 3 within the approximate limits of the force exerted by the ventricular strip. The limits of linearity were not studied and should be determined if the system is to be employed for the measurement of isotonic contractions.

The remainder of the apparatus differs from similar devices previously reported in several respects and is reported in brief. The muscle chamber consists of a cylindrical vessel 10 cm. in height and 4.5 cm. in diameter. As shown in Fig. 4, a polystyrene mercury-filled electrode with a platinum contact enters the chamber at the side arm through an ampul stopper. An air stone of the type used in aquariums is similarly introduced through an opening at the bottom of the bath. The muscle holder, as seen in Fig. 4 *A*, is likewise made of polystyrene plastic. In order to fix the muscle mechanically and electrically to the holder, the strip is first tied at both ends with silk thread. Nylon thread should not be used as it stretches. One end of the muscle is then pulled through a small, carefully bored hole approximately 0.5 mm. in diameter in a soft rubber-ampul cap. By stretching the hole with a forceps to enlarge the opening, approximately 1 to 2 mm. of the muscle is pulled into the inside of

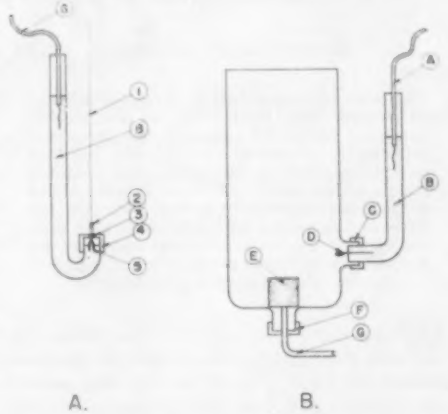

Fig. 4.—**A**, Muscle chamber. 1, Connection to lever arm; 2, muscle strip; 3, 0.5-mm. hole for insertion of strip; 4, rubber cap; 5, platinum cap; 6, connection to stimulator.

B, Electrode assembly. *A*, Connection to stimulator; *B*, mercury filled electrode; *C*, rubber cap; *D*, platinum electrode; *E*, air stone; *F*, rubber cap; *G*, oxygen supply.

the cap. The cap holding the muscle with approximately 1 cm. outside and 1 mm. inside the cap is then pushed down over the muscle holder in order that the muscle comes in contact with the electrode. The end of the muscle holder through which the electrode protrudes is expanded to such a size that the ampul cap fits very tightly. It is necessary to select an ampul cap having a sufficiently heavy top to avoid movement of the cap with each muscle contraction.

The muscle is stimulated four times per second with the output of a Tektronix type 162 waveform generator and a Type 161 pulse generator powered by a type 160A power supply. Such a system provides all the advantages of more expensive commercial biological stimulators and offers considerably more flexibility.

Temperature was controlled at 27° ± 0.25° by

the use of a Thermistemp temperature controller model 71 and a 2084 liquid immersion probe. Heating or cooling as required was accomplished by pumping water at a temperature differential of about 5° through 1/8-inch diameter polyethylene tubing wrapped around the muscle chamber.

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Studies on Bacterial Pyrogenicity II*

A Bacteriological Test for Pyrogens in Parenteral Solutions

By STANLEY MARCUS, CARL ANSELMO, and JOANNA LUKE

The basis and procedure for a culture method employing membrane filters to test freshly prepared parenteral solutions for pyrogenic capacity is presented. Under the limiting conditions described, solutions which yielded counts of less than 10,000 per L. were found to be nonpyrogenic by rabbit test. On the basis of laboratory and field trial, it is concluded that the bacteriological assay for the purpose described is as specific and sensitive as the rabbit assay for pyrogenicity.

WORK REPORTED (1, 2) has given support for the concept that official pyrogen tests, including the U. S. P. test, have a quantitative basis in terms of the numbers of bacteria required to induce a febrile response in rabbits. That is, a definite number of organisms must contaminate a solution before it becomes pyrogenic.

In this report a culture technique is described which has been successfully employed as an alternative for the official rabbit test for pyrogenicity of freshly prepared parenteral solutions. Results obtained are given and some implications concerning the method are explored.

MATERIALS AND METHODS

Pyrogen tests in rabbits were carried out with sterile (autoclaved) solutions according to the techniques described in the "U. S. Pharmacopeia XV," with minor exceptions previously noted (2). Interpretations of results were in accordance with U. S. P. XV. Samples of solutions to be tested by the culture procedure were taken before sterilization and filtered through so-called "membrane" filters (Millipore, type HA, white, grid, 47 mm.). That is, the batch of solution was prepared and either an aliquot was removed from the one sample or one or more containers of the bottled lot was sampled immediately prior to placing the remainder into the autoclave for sterilization. At least 100 ml. of each solution was filtered. Each filter was then aseptically transferred to a human (2–5%) blood agar plate and incubated at 37° to yield estimates of the numbers of living organisms present in the samples prior to sterilization. Colony counts were made after forty-eight hours incubation. Reasons for the use of the blood agar medium, incubation temperature, and time are considered in the discussion section.

Theoretical considerations as well as practical findings have led us to conclude that a freshly prepared solution yielding a count of 10,000 or less per L., under the defined conditions of the test, is nonpyrogenic as determined by rabbit assay.

RESULTS

Freshly distilled water, collected under ordinary conditions of cleanliness in the experimental laboratory from a glass still that condensed approximately 2 L. per hour, with no unusual precautions taken to avoid air-borne contamination, was usually sterile. Such water transferred to previously washed (detergent and hot tap water) and then steamed (live-steam hose) 5-gallon glass carboy containers maintained water in a nonpyrogenic state for the duration of the test period, that is, three months. The mouths of the carboys were covered by an initially sterile 1-L. Griffin beaker. After twelve to fourteen weeks three 5-gallon specimens prepared in this fashion yielded counts of 98, 174, and 35 organisms/L. The water in each carboy was converted to 0.9% NaCl by addition of the proper amount of a U. S. P. grade reagent. To simulate hospital laboratory conditions as well as mix the solution, the water was poured from the carboy into a

* Received September 18, 1959, from the College of Medicine, University of Utah, and Latter Day Saints Hospital, Salt Lake City.

Taken in part from a thesis submitted by C. Anselmo to the Graduate School of the University of Utah in partial fulfillment of the Master of Science degree.

This work has been aided by the American Sterilizer Foundation, Erie, Pa., and the Latter Day Saints Hospital Foundation for Medical Research.

10-gallon aluminum vat previously cleaned and then rinsed with freshly distilled water. Average plate counts after forty-eight-hours incubation on two 100-ml. samples of the finished products were 34, 62, and 49 organisms, corresponding to values of 340, 620, and 490 organisms/L. Immediately after samples were taken for bacterial counts, liter amounts were sterilized by autoclaving. Pyrogen tests in rabbits with the autoclaved samples were negative.

Freshly distilled water, collected under usual conditions of cleanliness in the central supply area of a large general hospital, was found to be sterile by membrane filter technique. This water, prepared from a still operating by connection to a steam line, was collected into a 12-gallon glass carboy. A plastic hose through which water was taken from the carboy was found to be consistently contaminated with organisms of the family *Achromobacteriaceae*. These organisms, which are not significant pyrogen producers (2), were reduced to low numbers by flushing about 2 L. of freshly distilled water through the plastic drain after washing the terminal portion of the drain with detergent in hot tap water. The tap water was not softened by ion exchange resin treatment. Freshly distilled water from this carboy was used to prepare small (4-L.) batches of 0.9% NaCl (4 batches), 5% dextrose (4 batches), or a mixture of these substances (2 batches) in a 6-L. flask; all these preparations but one contained less than 100 viable organisms/L. and all were negative in the rabbit pyrogen assay. The chemicals used in these preparations were U. S. P. grade.

Water from a Barnstead electrically powered, all metal still, rated at one gallon per hour, was collected in 5-gallon glass carboys and found to be sterile on repeated tests by membrane filter analysis. The occasional presence of one or two colonies in cultures of membrane filters through which liter amounts had been sampled was interpreted to represent transfer contamination. This water was used to rinse out the 10-gallon aluminum vat previously referred to. Following rinsing, 4 gallons of water was added to the vat and converted to 0.9% saline solution by the addition of 36.2 Gm. of U. S. P. NaCl. From the hose outlet at the bottom of the vat, successive 500-ml. samples yielded colony counts of 3, 4, and 2 organisms corresponding to estimates of 6, 8, and 4 organisms per L. The autoclaved specimens were negative for pyrogens by rabbit test. These data are summarized in Table I.

A critical evaluation of the bacteriological test for pyrogens in freshly prepared solutions was carried out in cooperation with a reputable company which prepares and sells parenteral solutions.[1] Samples at the plant were collected at or from: (*a*) the distilled water delivery outlet of the main still, (*b*) a collecting tank containing approximately 50 gallons of freshly distilled water, (*c*) this latter source after mixing in the U. S. P. grade chemicals, (*d*) the washed, filled, and capped 1-L. bottles ready to be autoclaved. It was predicted that the successive samples would yield cumulative colony counts. In general, this was found to be true.

[1] We are grateful for the cooperation of the Allied Pharmacal Co., Centerville, Utah.

TABLE I.—RELATION BETWEEN BACTERIAL COUNT AND PYROGEN TEST RESULTS IN VARIOUS PARENTERAL SOLUTIONS

Sample	Membrane Filter Count Colonies/L.	U. S. P. Rabbit Pyrogen Test
Glass distilled water		
Fresh	0	..
No. 1 5 gals. After 14 wks.	<100	..
No. 2 5 gals. After 13 wks.	<200	..
No. 3 5 gals. After 12 wks.	<100	..
No. 1 Converted to saline	<400	neg.
No. 1 Converted to saline	<400	neg.
No. 2 Converted to saline	<700	neg.
No. 3 Converted to saline	<500	neg.
Steam line distilled water		
Fresh (4-L. Batches)	0	..
No. 4 0.9% Saline	<100	neg.
No. 5 0.9% Saline	< 10	neg.
No. 6 0.9% Saline	< 10	neg.
No. 7 0.9% Saline	< 10	neg.
No. 8 5% Dextrose	<100	neg.
No. 9 5% Dextrose	<100	neg.
No. 10 5% Dextrose	<100	neg.
No. 11 5% Dextrose	<100	neg.
No. 12 5% Dextrose in saline	<200	neg.
No. 13 5% Dextrose in saline	<100	neg.
Barnstead distilled water		
Fresh (4-gal. Batch)	0	..
No. 14 0.9% Saline	< 10	..
	< 10	neg.
	< 10	..

For example, in one experiment, the fresh distillate was sterile, the collected distillate contained an estimated 50–100 organisms/L., the 5% dextrose solution, following mixing, yielded an estimate of 440 organisms/L., and two 1-L. bottles selected at random from the 180-L. bottle batch yielded estimated counts of 560 and 680 organisms/L. Samples of autoclaved specimens were sterile and pyrogen tests were negative. Inconsistencies in counts in 12 experiments were ascribed to logistic problems, that is, collecting and bringing the samples to the laboratory to be tested. In any event, in these experiments, as well as another 62 experiments in which cultures were carried out on samples from bottles taken from racks just prior to placing in the autoclave, counts of 240 to 3,400 organisms/L. were obtained. The distribution of these counts, in estimated number of organisms per L., is shown in Table II. The specimens included examples of the usual commercially available parenteral solutions of the saline, dextrose, and Ringer's types. All batches yielded negative pyrogen tests in rabbits.

DISCUSSION

Since bacteriological culture tests have been indicted as unreliable for general pyrogen assay in the past (3–7) it is necessary to consider in detail the hypotheses and arguments on which are based this claim for the reliability of a culture method.

If it is recalled that all assay tests are standardized with both explicit (defined) and implicit

TABLE II.—DISTRIBUTION OF COLONY COUNTS ON 74 FRESHLY PREPARED SOLUTIONS PRIOR TO STERILIZATION

Colonies Counted[a] per 100-ml. Sample	Organisms Estimated, per L.	No. of Specimens
24–50	240–500	2
> 50–<100	> 500–<1000	21
>100–<150	>1000–<1500	27
>150–<200	>1500–<2000	14
>200–<250	>2000–<2500	6
>250–<300	>2500–<3000	1
>300–<350	>3000–<3500	3

[a] Blood agar, 48 hours at 37°.

("common-sense") limits of error, then the defense of the proposed assay for pyrogens in freshly prepared parenteral solutions is more readily rationalized.

By "parenteral solution" we refer to any of the usually available salt and simple sugar solutions put up in volumes of 1 L. to as little as 5 ml. and designed for intravenous or other form of injection or infusion into man. For the present we specifically exclude consideration of vitamin, antibiotic, and other biological preparations which are administered by injection.

The object of the assay method proposed is to determine with confidence if a freshly prepared solution contains less than 10 organisms per ml. (10,000/L.) by the technique described. Under these circumstances, thermophilic, psychrophilic, photosynthetic, and anaerobic bacteria will not be detected. Fastidious pathogens such as *N. meningitilis*, *H. pertussis*, or *M. tuberculosis* will not grow out. Of course, nonviable bacteria as well as other forms of microbic life (viruses and protozoa) will also go undetected. The hypothesis explored therefore is that salt and sugar solutions prepared for parenteral use, by prescribed standard methods, with freshly distilled water, will be nonpyrogenic if such solutions contain fewer than 10,000 bacteria, yeast, or mold spores per L., without respect to species of such microorganisms, when tested immediately after preparation by the method described.

Reasons for the explicit limitations to the method are derived mainly from material previously reported (1, 2). It has been found that at least 1,000 per ml. of the most pyrogenic bacteria must contaminate a solution, that is then sterilized by autoclaving, before it will yield a pyrogenic response by rabbit test. Since the potent pyrogen producers are apparently rare as compared to the usual contaminants of freshly prepared parenteral solutions, the hypothesis may be suggested that defining a count of 10 organisms per ml. (10^4/L.), rather than 1,000 per ml. (10^6/L.), as potentially pyrogenic results in a "safety factor" of about 100 times to account for nonviable pyrogenic sources in the material tested. In other words, a solution yielding a count of 10^4 to 10^5 added organisms per L. will be negative in the rabbit test. This will be true if the organisms are added, but not necessarily so if the organisms grow in the solution. This is why the test must be restricted to freshly prepared solutions.

We delineate a "freshly prepared solution," at present, as one prepared with water distilled, used, and sterilized within a twenty-four-hour working period. The twenty-four-hour working period has been chosen arbitrarily both on the basis of trial and from theoretical considerations. Water collected overnight in previously cleansed tanks and then used for washing and solution preparation on the following day has been found to be indistinguishable by rabbit test from water collected and used during an eight-hour period. This is not surprising when we view the fact in the light of the hypothesis that difficulty with pyrogens arises from organisms added to the solution during the manufacturing process and prior to sterilization. Conclusions of this nature were reached by Seibert (8) who stated: "... there seems to be no doubt ... that the pyrogen is a bacterial contaminant, as suspected by many former investigators .... It is possible to free distilled water of this contamination by a proper distillation and only such fresh distillates should ever be used in the preparation of solutions for parenteral injections. If these fresh distillates are sterilized immediately and preserved sterile they will remain free from pyrogen. On the other hand, if they are once allowed to become contaminated, it is not possible to free them of pyrogen by autoclaving ...." In concurring with this conclsuion we amend the statement by defining "fresh distillates" as we have done and by introducing the qualitative and quantitative concepts of pyrogenicity for freshly prepared solutions. In our experience, the most frequent contaminants of distilled water (*Achromobacteriaceae*) have a low pyrogenic potential (2) and prolonged generation time under natural conditions. Also, in this regard, the dangerous heterotrophic species of *Enterobacteriaceae* do not find freshly distilled water, containing trace amounts of atmospheric gases in solutions, a choice medium should they be inoculated into the distilled water during the distillation period; and there is not sufficient time for significant numbers of organisms to accumulate in the prepared solution before it is sterilized if the working period is restricted to twenty-four hours. Of course, stills, receptacles, containers, and delivery lines must be of types which are easily and thoroughly cleaned so that no debris may be trapped anywhere to act as a culture inoculum or medium.

The hypothesis that each colony that grows out arises from a single bacterial cell is drawn from experimental observation of the satisfactory relationship between organisms added to solutions and membrane filter counts made from aliquots of these solutions.

The assumption that all microorganisms are equally pyrogenic is entirely unjustified, does not fit the available data, and is employed on the basis of being a further safety factor in the allowable count; that is, organisms that we have isolated from freshly prepared parenteral solutions, immediately prior to autoclaving, are much lower in pyrogenic potential than is indicated by the assay limits of acceptability.

It should be apparent that the proposed method is unsuitable for use with ion exchange water. Until such time that suitable methods are found for readily cleaning and sterilizing ion exchange resin columns the use of such water for parenteral preparations should be discouraged rather than guarded. At this time, the use of ion exchange water for

parenteral preparations is forbidden in U. S. P. procedure and lack of available information on the subject makes use of such water hazardous from all points of view.

The bacteriological assay procedure proposed should monitor the cleanliness of the plant operation. That is, if a normal range of bacterial counts is once established, a significantly increased count deviation from this mean should indicate the existence of an actual or potential trouble source.

Disadvantages of the procedure are apparent. A bacteriology laboratory or semblance thereof must be available. The test is a secondary type procedure based on the primary bioassay with rabbits, which itself is fallible. The time limitations of the test have been stressed, that is, unlike the rabbit test, the culture assay cannot be delayed but must be performed as soon as the solution is prepared and is ready for autoclaving. Furthermore, the results cannot be determined for forty-eight hours.

SUMMARY

A simple culture technique, employing membrane filters (Millipore, HA) to test freshly prepared parenteral solutions for pyrogenic capacity, has been described. The basis and limiting conditions for the method are noted and discussed, as are certain advantages and disadvantages.

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A Comparative Study of Theophylline Preparations: Digestive Absorption*

By SAMUEL H. WAXLER and HERBERT B. MOY

Six theophylline preparations have been tested for their absorption from the gastrointestinal tracts of animals as reflected by blood theophylline levels. These values were obtained first from the stomach, and then from the remainder of the gastrointestinal tract. The results showed satisfactory absorption with all medications, and the wide variation inherent in oral preparations. No real superiority was evident in the six preparations when the available theophylline was the same in each drug.

In 1949 Schack and Waxler (1) described an ultraviolet spectrophotometric method for the determination of theophylline in blood and tissue. Subsequently this method was applied to a study on groups of patients and blood theophylline levels were determined following the administration of aminophylline by intravenous, intramuscular, oral, rectal, and inhalation routes (3, 4, 5). Attention was paid primarily to the rate of appearance, the level attained, and the disappearance of the drug from the blood stream. Since that time, several papers on new oral theophylline preparations have appeared. Two points are generally stressed with these preparations: the superior blood levels, and the fewer gastrointestinal disturbances. The problem of absorption of the drug from the gastrointestinal tract has been considered only in very general terms. Since little is known, no references are made to the differential absorption of the drug from any particular part of the tract, i. e., the stomach or the small intestine. This present study is an attempt to determine in animals the amount of absorption of theophylline from the isolated stomach in a set interval of time, and also the absorption which occurs from the intestinal tract after the theophylline material leaves the stomach.

* Received October 27, 1959, from Stanford University, School of Medicine, San Francisco, Calif.

MATERIALS AND METHODS

In these experiments six theophylline preparations: theophylline (Searle); aminophylline (Searle); calcium theophyllinate (Searle) (2); aminophylline-aluminum hydroxide-ethyl aminobenzoate (Irwin, Neisler); aminophylline-aluminum hydroxide gel (Massengill); and choline theophyllinate (Barnes Hind); were examined for their absorption, first from the stomach of rabbits and dogs, and, subsequently, from the remainder of the intestinal tract of these animals. All of the preparations

were given so that an equal amount of available theophylline was used. The total theophylline in each compound was computed, and this was given in the amount of 15 mg. of theophylline per Kg. of body weight to the rabbits, and 25 mg. of theophylline per Kg. to the dogs.

Forty-one rabbits weighing between 2 and 3.4 Kg. were taken off feed twelve hours before the experiments. Under tribromoethanol and light ether anesthesia, a small abdominal incision was made, the stomach exposed, and a permanent ligature placed at the distal end of the stomach. The prescribed amount of preparation was administered and allowed to enter the stomach.

Thirty-eight rabbits in the same weight range were prepared in a similar manner under tribromoethanol and light ether anesthesia. A soft rubber-shod clamp was temporarily placed at the distal end of the stomach and the animal was given, by stomach tube, one of each of the six above-mentioned theophylline preparations. At the end of one-half hour the clamp was removed and the material allowed to continue into the small intestine.

A similar experiment was performed with dogs. The animals were anesthetized with chloralose, 100 mg. per Kg. of body weight subcutaneously, and kept under light ether anesthesia as required. The abdominal cavity was opened, a clamp was placed at the distal end of the stomach, and the various theophylline preparations, 25 mg. per Kg., were administered by stomach tube. The clamp was removed one hour after the administration of the drug.

A preliminary blood sample for "blank" determination was drawn prior to the administration of the drug; then serial samples were drawn through the next five to seven hours. The blood samples were analyzed for theophylline content by the method of Schack and Waxler. A Beckman DU ultraviolet spectrophotometer was used for determination of the values.

RESULTS

The data obtained following oral administration of theophylline preparations to rabbits with a ligature at the distal end of the stomach are shown in Table I. The significant degree of absorption from the stomach after thirty minutes is reflected in the elevated blood levels. These levels were maintained and increased over the next seven hours. The range within each group is quite marked; and these variations are noted with all drugs tested.

The second phase of this experiment gives the results following temporary occlusion of the distal end of the stomach of the rabbit with a rubber-shod clamp which is removed thirty minutes after oral administration of the drug. The values at the thirty-minute mark (Table II) indicate theophylline levels obtained from absorption from the stomach

TABLE I.—BLOOD THEOPHYLLINE LEVELS OBTAINED FROM ABSORPTION OF THEOPHYLLINE PREPARATIONS FROM THE STOMACH OF RABBITS[a]

	Time			
	30 Minutes	1 Hour	3 Hours	7 Hours
Theophylline	110 ± 39	169 ± 83	347 ± 70	521 ± 96
Calcium theophyllinate	112 ± 52	145 ± 57	291 ± 49	473 ± 45
Aminophylline	127 ± 62	178 ± 67	404 ± 105	474 ± 64
Aminophylline-Al(OH)$_3$ gel	106 ± 21	170 ± 54	386 ± 91	579 ± 115
Aminophylline-Al(OH)$_3$-ethyl aminobenzoate	109 ± 57	174 ± 81	313 ± 76	472 ± 106
Choline theophyllinate	123 ± 38	243 ± 61	370 ± 56	519 ± 86

[a] Theophylline mcg./100 cc. of blood.

TABLE II.—BLOOD THEOPHYLLINE LEVELS OBTAINED FROM ABSORPTION OF THEOPHYLLINE PREPARATIONS FIRST FROM THE STOMACH AND THEN FROM THE REMAINDER OF THE GASTROINTESTINAL TRACT OF RABBITS[a]

	Time			
	30 Minutes	1 Hour	3 Hours	7 Hours
Theophylline	115 ± 75	172 ± 93	318 ± 59	587 ± 180
Calcium theophyllinate	57 ± 28	161 ± 79	414 ± 79	580 ± 119
Aminophylline	93 ± 49	144 ± 35	428 ± 54	688 ± 149
Aminophylline-Al(OH)$_3$ gel	93 ± 30	148 ± 31	282 ± 20	560 ± 182
Aminophylline-Al(OH)$_3$-ethyl aminobenzoate	135 ± 23	340 ± 220	424 ± 182	827 ± 216
Choline theophyllinate	157 ± 36	319 ± 14	654 ± 127	724 ± 130

[a] Theophylline mcg./100 cc. of blood.

TABLE III.—BLOOD THEOPHYLLINE LEVELS OBTAINED FROM ABSORPTION OF THEOPHYLLINE PREPARATIONS FIRST FROM THE STOMACH AND THEN FROM THE REMAINDER OF THE GASTROINTESTINAL TRACT OF DOGS[a]

	Time					
	30 Minutes	1 Hour	2 Hours	3 Hours	4 Hours	5 Hours
Theophylline	420 ± 41	508 ± 110	1044 ± 190	1238 ± 258	1591 ± 343	1715 ± 345
Calcium theophyllinate	507 ± 383	715 ± 465	1145 ± 398	1483 ± 531	1618 ± 385	1495 ± 174
Aminophylline	545 ± 88	905 ± 134	1569 ± 408	1976 ± 538	2320 ± 450	2449 ± 418
Aminophylline-Al(OH)$_3$ gel	530 ± 86	1045 ± 96	1215 ± 432	1549 ± 582	1670 ± 626	1662 ± 604
Aminophylline-Al(OH)$_3$-ethyl aminobenzoate	760 ± 400	1222 ± 456	1227 ± 462	1998 ± 755	2061 ± 880	1962 ± 826
Choline theophyllinate	654 ± 225	853 ± 298	1410 ± 248	1728 ± 294	1955 ± 429	2085 ± 403

[a] Theophylline mcg./100 cc. of blood.

alone; the subsequent levels are obtained from the entire gastrointestinal tract over a period of six and one-half hours. The results here, as in Table I, show a wide spread of values, and no group is consistently superior.

We repeated this same experiment using dogs, varying only in the increased dose of medication and obstruction of the stomach for a shorter period. The clamp at the distal end of the stomach was removed at the end of one hour and the experiment allowed to continue for five hours. There is likewise significant absorption from the dog stomach during the first hour which continues from the remainder of the gastrointestinal tract. The results, as shown for the six drugs in Table III, appear to fall in the same pattern as the preceding experiments (Tables I and II).

DISCUSSION

In previous experiments with hospitalized patients we showed that aminophylline tablets were readily absorbed. By the first hour all persons so tested had a definite blood theophylline level. However, with this dosage given the values ranged from 100 to 500 mcg. per ml. of blood.

In this experiment with rabbits and dogs there is a similarly satisfactory degree of absorption with all drugs tested. There is also the wide spread of levels within the groups using the same brand of preparation.

There is no apparent superiority when the preparations under test are given with the same theophylline content. Clinically it has been observed that the various combinations and modifications of aminophylline give no great superiority over the basic material. No real therapeutic advantage can be claimed for the various additives.

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Notes

Note on the Alkaloids of *Methysticodendron amesianum**

By IRWIN J. PACHTER and ALICE F. HOPKINSON

HIGH in the Colombian Andes, in the geographically isolated valley of Sibundoy, grows a rare and beautiful narcotic tree employed by the Inga and Kamsá Indians in their witchcraft. The tree, *Methysticodendron amesianum*, was described in 1955 by Professor R. E. Schultes (1) who held that it is a member of a hitherto undescribed genus of the *Solanaceae*. The Indians classify the plant together with other intoxicating solanaceous plants of the region, but find that it is even more potent and dangerous than the tree species of *Datura*. A description of the intoxication indicates that the principles are alkaloids of the tropane series (1).

In the hope that this unexplored genus might yield new tropane alkaloids of greater potency and utility than those known, supplies of the plant were obtained from Colombia for study.[1]

Prolonged extraction with hot ethanol caused extensive decomposition of the alkaloidal principles. It is of interest, in this connection, that the Indians prepare infusions of the leaves with cold water, heating slightly just before administration. The liquid is never allowed to boil (1).

Continuous extraction with cold ethanol followed by concentration *in vacuo* and standard processing yielded crude alkaloidal material in 0.3% yield. Paper chromatography showed the bases to consist of one very major alkaloid and three minor alkaloids.

The total alkaloid mixture was tested pharmacologically and was found to produce biological effects which were practically identical, both quantitatively and qualitatively, with those produced by *l*-scopolamine.

Treatment of the mixture with picric acid in methanol yielded *l*-scopolamine picrate from which the pure base was generated. The alkaloid was identified through comparison of its infrared spectrum and its hydrobromide, hydriodide and methiodide derivatives with those of authentic *l*-scopolamine.

From the picrate mother liquors, mixed residual bases were isolated. Paper chromatography indicated that the same four bases were present, *l*-scopolamine and one other now being predominant.

The second predominant alkaloid proved to be atropine. It was not isolated but was identified through comparison of its R_f values in three solvent systems with those of atropine.

The two minor bases were not identified.

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[1] The authors are grateful to Professor R. E. Schultes of Harvard University, Cambridge, Mass., and Dr. J. M. Idrobo of Bogota, Colombia, for supplies of authentic plant material.
Note added in proof. After this manuscript was submitted, Professor Schultes informed us that a thesis on *Methysticodendron amesianum* has been written by J. F. Theilkuhl of the Universidad Nacional de Colombia.

l-Scopolamine constitutes over 80% of the alkaloidal content of *Methysticodendron amesianum*. If it is correct that the alkaloids of this plant are more potent than those of the *Datura* species in producing the desired frenzy and narcosis in Indians, it may be the result of the high ratio of scopolamine to atropine. Scopolamine may produce excitement, hallucinations, and delirium, even at therapeutic doses, whereas with atropine, doses bordering on the toxic are generally required before hallucinations and central excitation are observed (2).

EXPERIMENTAL

A 13-Kg. sample of dried ground leaves and stems of *Methysticodendron amesianum* was extracted continuously with ethanol at room temperature until the marc was exhausted of alkaloids. The extract was evaporated to dryness *in vacuo* at 40° and the residual material was stirred with 1.5 L. of 2% tartaric acid for three hours. The aqueous and organic layers were separated by centrifugation. The procedure was repeated six times with fresh 1.5-L. portions of 2% tartaric acid. The combined aqueous solutions were washed with 1 L. of ether, made basic with ammonia, and extracted with chloroform until exhausted of alkaloids. The chloroform extract was dried over magnesium sulfate and evaporated to dryness. It was extracted with ether and all but 4 Gm. of dark resinous matter dissolved. Evaporation of the ether left 38 Gm. of crude total bases. Paper chromatography in 10:1:10 butanol:acetic acid:water showed the bases to consist of one very major alkaloid (R_f 0.60) and three minor alkaloids (R_f 0.35, 0.69, and 0.81). The bases were dissolved in 200 ml. of methanol and treated with 45 Gm. of picric acid in 400 ml. of methanol. A crystalline picrate, 41 Gm., m. p. 190°, was obtained. Upon recrystallization from acetone, it melted at 193°.

Anal.—Calcd. for $C_{17}H_{21}NO_4 \cdot C_6H_3N_3O_7$: C, 51.88; H, 4.54; N, 10.52. Found: C, 51.96; H, 4.74; N, 10.43.

A sample of alkaloid, $[\alpha]_D^{25}$ −24.5 (H_2O), was generated from the picrate and converted into a hydrobromide, m. p. 193°; a hydriodide, m. p. 200–201°; and a methiodide, m. p. 215–217°. The derivatives proved to be identical with those prepared from authentic *l*-scopolamine.

After removal of crystalline *l*-scopolamine picrate, the picric acid mother liquors were concentrated and 6 Gm. of picric acid crystallized and was removed. The solution was then evaporated to dryness and the residue partitioned between dilute aqueous ammonia and chloroform. The chloroform solution was concentrated and extracted with 2 *N* hydrochloric acid. The acid layer was basified with ammonia and the alkaloids were re-extracted with chloroform. Evaporation of the latter left 5.8 Gm. of mixed alkaloids.

Paper chromatography showed that two major and two minor bases were present, one of the major components still being *l*-scopolamine. The second major component was found to be atropine. Although not isolated and characterized, its nature was determined by comparison of R_f values of authentic atropine with those of the mixed alkaloids in 10:1:10 butanol:acetic acid:water, 10:1:10 isoamyl alcohol:formic acid:water, and 11:2:10 isoamyl alcohol:formic acid:water.

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Note on the Effect of Organic Nitrogen Solvents Upon Specific Rotation*

By JOSEPH ROSIN and C. J. WILLIAMS

Tabular data are presented to show that the use of organic nitrogen compounds, and particularly quinoline, may be employed as solvents to enhance the optical rotation of various compounds, thus increasing the accuracy in their identification or assay.

MANY NATURALLY occurring organic compounds cause rotation of a plane of polarized light. Nearly all sugars, their related carboxylic acids, and most alkaloids have this property. In addition, several of the vitamins: ascorbic acid, calciferol, cyanocobalamin, and riboflavin, as well as the large group of steroids including the therapeutically important estrones, cortisone and its derivatives exhibit this phenomenon.

Specific rotation is valuable not only for identification, but also for quantitative assay. It serves as a basis for the estimation of the pure sucrose content in raw sugar. Dextrose (glucose) injections, so extensively used medicinally, are assayed by means of optical rotation. The content of quinine in cinchona bark is universally determined from its optical rotation under specific experimental conditions.

The angle of rotation and thus the specific rotation, varies with the conditions under which the measurement is made. Among the factors having the greatest bearing is the solvent. The solvent not only affects the degree of rotation, but it even may reverse the direction of the rotation; from right (+) to left (−), and vice versa.

In all quantitative measurements it is axiomatic that the greater the quantity or number of units measured the greater is the accuracy. For a more

* Received March 20, 1960, from Merck & Co., Inc., Rahway, N. J.

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TABLE I.—SPECIFIC ROTATION OF SOME ORGANIC COMPOUNDS IN ORGANIC SOLVENTS

	Nonnitrogen Solvents									
	Aniline	n-Butyl amine	Monoethanol-amine	Isoquinoline	Pyridine	Quinoline	Alcohol	Chloroform	Water	Dioxane
Aconitine	− 47°	..	..	− 97°	− 34°	− 86°	+ 18°[a]	+ 18.5°[a]	..	..
Ascorbic acid	+ 22°	+154°	+153°	+154°	+103°	+312°	+ 57°		+ 21.5°[a] +NaOH or NH₄ +119°	+ 63°
Brucine anhydrous	− 80°	−132°	−125°	−124°	−130°	−160°	− 84°[a]	−118°[a]	..	..
Brucine sulfate[f]	− 10°	..	..	− 36°	− 61°	− 72°	..	..	− 27°	..
Calciferol (Vitamin D_2)	..	..	..	..	+100°	+138°	+106°[a]	..	..	..
Cholesterol	− 39°	− 34°	..	..	− 32°	− 31°	..	− 39°[a]	..	+ 36°
Cholic acid	+ 22°	+ 38°	+ 32°	+ 29°	+ 35°	+ 65°	+ 37°[a]	..	..	+ 33°
Cinchonidine	− 92°	..	..	− 35°	− 51°	− 28°	+109°[a]	..	..	..
Cinchonine	+152°	..	..	+129°	+134°	+118°	+229°[a]	−210°[a]	..	..
Cocaine	− 51°	− 22°	− 24°	− 52°	− 47°	− 55°	− 35°[a,b]	− 16°[a]	..	..
Cocaine hydrochloride	−105°	− 21°	− 17°	− 50°	− 49°	− 65°	..	..	− 72°[a]	..
Codeine, anhydrous basis	−162°	−158°	−166°	−218°	−212°	−203°	−144°[a]	−119°[a]	−114°[a]	−143°
Colchicine	−200°	−202°	..	− 65°	−139°	[c]	..	−121°	−423°[a]	..
1-Dehydrocholesterol activated (Vitamin D_3)	..	..	..	..	+106°	+163°	+109°	..	..	..
Dehydrocholic acid	..	..	..	+ 46°	+ 51°	+ 89°	..	..	+NaOH −29°	+ 31°
Digitoxin	..	About +6°	About +8°	− 20°	− 10°	− 15°	..	+ 18°	..	approx. +5°
Ephedrine anhydrous	− 8°	− 20°	− 14°	− 29°	− 23°	− 24°	approx. −5°		+ 12.5°	..
Ephedrine hydrochloride	− 38°	..	..	− 35°	− 67°	−141°	..	..	+ 34°[a]	..
Estrone	+132°	+170°	+116°	+111°	+147°	+103°	..	..	..	+166°[a]
Menthol	− 42°	− 50°	− 42°	− 56°	− 47°	− 66°	− 49°[a]	..	..	..
Morphine[f]	−141°	..	..	−178°	−186°	−188°	−149°[a,d]	−140°[a]	..	−123°
Morphine sulfate[f]	..	..	..	−136°	−143°	−138°	..	..	..	−109°[a]
Oubain anhydrous	− 24°	..	..	− 10°	− 47°	+ 13°	..	..	− 32°[a]	..
Pilocarpine hydrochloride	+102°	+ 40°	+ 18°	+123°	+ 96°	+156°	+ 79°	..	+ 90°[a]	..
Pilocarpine nitrate	..	..	..	+108°	+ 91°	+138°[a]	..	..	+ 81°[a]	..
Quinidine anhydrous	+196°	+238°	+232°	+118°	+156°	+167°	+255°[a]	+230°[a]	..	..
Quinine[f]	−116°	−134°	..	− 42°	− 67°	− 86°	−167°[a]	−118°[a]	..	..
Quinine hydrochloride[f]	− 59°	−113°	..	− 20°	− 68°	− 27°	..	..	−156°[a]	..
Santonin	−113°	− 38°	..	−162°	−170°	−168°	−174°	..	..	..
Strychnine	− 86°	..	..	−127°	−158°	−156°	−104°[a]	−140°	..	..
Strychnine sulfate[f]	..	..	..	− 61°	− 95°	− 98°	..	..	− 28.5°	..
Sucrose	..	+74°	+ 66°	+115°	+ 83°[a]	+ 70°	..	..	+ 66°[a]	..

[a] Value given in the literature.
[b] Solution of 50% alcohol.
[c] When the rotation in quinoline solution was measured shortly after the solution was prepared it corresponded to +15° to +18° specific rotation, but on standing overnight, rotation was completely abolished. In isoquinoline solution, however no change in the rotation was evidenced even after standing for twenty-four hours.
[d] Methanol.
[e] Measurement made within thirty minutes after the solution was prepared, as the latter became green on longer standing.
[f] Determination on anhydrous basis.

accurate determination of specific rotation, therefore, it is desirable to use a quantity of the substance under examination that will give a reasonably high instrument reading. For the estimation of sucrose content in crude sugar a concentration of about 25% is used. However, use of a high concentration may be limited by the solubility of the substance, and in research work especially, by the availability of the material. To enhance the rotation, therefore, the analyst will often try other solvents.

The solvents ordinarily employed are water, alcohol, dioxane, and chloroform. The use of pyridine as a solvent for optical rotation measurements has been reported occasionally in the literature and in two or three instances it is now used in the United States Pharmacopeia for this purpose.

Investigation of other nitrogenous solvents was felt desirable. One of the first such solvents employed was quinoline. Determination of the specific rotation of ascorbic acid in this solvent gave a value fourteen times that noted with water as a solvent. With this encouraging result a series of thirty-two compounds was studied. In addition to quinoline other organic nitrogen solvents were employed; these included isoquinoline, pyridine, aniline, monoethanolamine, and *n*-butylamine.

EXPERIMENTAL

Depending on the solubility, 40 to 200 mg. of the finely powdered substance was placed in a 10-ml. volumetric flask, and 8–9 ml. of the solvent was added. Alkaloid bases whose quinoline or isoquinoline salts are soluble in the respective solvents

dissolve readily, but the hydrochlorides or sulfates of the alkaloids and sugars dissolve quite slowly. In these cases the flask was shaken in a mechanical shaker for a few hours, and if necessary warmed to 50–60° in order to achieve complete solution. The solution was then brought to volume and mixed. Because of their lower viscosity, solution was more rapid in pyridine and the amino alcohols than in quinoline or isoquinoline. The solutions should be protected from strong light during preparation and handling, and should not be allowed to stand for longer than twenty-four hours before taking the rotation, otherwise oxidation of the solvent, with subsequent darkening, will result.

The result of this study is presented in Table I. Of the 32 compounds and 10 solvents studied, at least twelve of the solutes exhibited the highest rotation in quinoline, and five in isoquinoline. The effect of quinoline on the rotation of ascorbic acid and of colchicine is most striking. The rotation of the former is magnified fourteenfold as compared with that in water, while the high specific rotation of colchicine (about 425°) in aqueous solution is reduced, in quinoline, to less than one-twentieth of this value.

Communication to the Editor

Color Selection for Uncoated Tablets*

Sir:

Selection of acceptable colors for uncoated tablets is difficult for several reasons: (*a*) It is difficult to obtain uniquely colored tablets unless two or more colorants are blended. This blending process is rather haphazard and very time-consuming. (*b*) Many of the approved colorants do not exhibit desirable light stability. (*c*) The status of several commonly used FD&C and D&C dyes and pigments is uncertain.

With these limitations in mind, it was desirable to prepare a tablet color bank using light-stable pigments whose status is unquestioned. Since red, blue, and yellow are the primary colors, it is theoretically possible to obtain any desirable hue by blending these colors. Thus, it was reasoned that three colored granulations could be used to prepare tablets of any desired hue. Because only light-stable pigments would suffice, the following D&C colors were selected: Blue No. 6, Red No. 35, and Yellow No. 11 (1).

Three separate wet granulations of these primary colors were prepared containing 0.1 per cent blue, 0.1 per cent red, and 0.2 per cent yellow pigments. Sieve analyses showed that 92 per cent of these colored granulations passed through a 100-mesh screen (2).

A number of small batches of tablets containing varying ratios of two of the three primary granulations were prepared. Pigment ratios varied as follows: yellow 1 part:red $^1/_{48}$ to 4 parts, red 1 part:blue $^1/_{24}$ to 4 parts, and yellow 1 part:blue $^1/_{48}$ to $2^1/_2$ parts. All of these combinations gave evenly colored tablets with different hues. Tablets with less intense colors were also prepared by diluting the blended granulations with noncolored ones. In some cases the dilution was 1:20.

This approach is useful for preparing a rainbow of pastel colored tablets. When a hue has been selected, subsequent tablet batches can be prepared by combining the pigments in the desired quantities and granulating in the conventional manner.

(1) Tucker, S. J., Nicholson, A. E., and Engelbert, H., THIS JOURNAL, **47**, 849(1958).
(2) Tucker, S. J., and Hayes, H. M., *ibid.*, **48**, 362 (1959).

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Book Notices

The Neurochemistry of Nucleotides and Amino Acids. Edited by ROSCOE O. BRADY and DONALD B. TOWER. John Wiley & Sons, 440 Fourth Ave., New York 16, N. Y., 1960. xii + 292 pp. 14.5 × 23 cm. Price $10.

This book covers the symposium of the Section on Neurochemistry of the American Academy of Neurology held April 24–25, 1958. Papers are followed by discussions, with a general summary discussion. Author and subject indexes are appended.

Staining Procedures. 2nd ed. Revised by H. J. CONN, MARY A. DARROW, and VICTOR M. EMMEL. The Williams & Wilkins Co., 428 East Preston St., Baltimore 2, Md., 1960. xii + 289 pp. 15 × 23 cm. Paperbound. Price $5.

This is an authoritative compilation of staining procedures used by the Biological Stain Commission, University of Rochester Medical Center. The procedures are not intended to be considered as standard or official but they are presented in a standardized style for the convenience of users. A given procedure is frequently one of a number of ways of obtaining good staining results. However, one can rely on the tested methods described. A bibliography and subject index are appended.

Nucleoproteins. Edited by R. STOOPS. Interscience Publishers, Inc., 250 Fifth Ave., New York 1, N. Y., 1960. 364 pp. 16 × 24.5 cm. Price $10.50.

This book records the proceedings of the eleventh Solvay conference on chemistry held June 1–6, 1959, at Brussels, Belgium. Paper titles are: Le rôle biologique des acides nucléiques, Molecular structure of deoxyribonucleoproteins, Constitution of histones, Les propriétés physiques de l'acide désoxyribonucléique en solution, Heterogeneity of nucleic acids and effects of chemical and physical agents, Nucleic acids of microorganisms, Biosynthesis of ribonucleic acid, Formation of helical polynucleotide complexes, Structure of RNA containing viruses, and Chemical synthesis of polynucleotides. Each paper is discussed and a general discussion is included. No index.

Radioactivity for Pharmaceutical and Allied Research Laboratories. Edited by ABRAHAM EDELMANN. Academic Press, Inc., 111 Fifth Ave., New York 3, N. Y., 1960. xii + 171 pp. 13 × 20.5 cm. Price $6.

This little book includes the papers presented at a symposium sponsored by Nuclear Science and Engineering Corporation. Subjects discussed include: Effects and utilization of ionizing radiation; Principles, methods, and areas of usefulness of radioactivity in the pharmaceutical and allied sciences: assays, product development, drug absorption, and excretion; and Radiobiochemistry in the pharmaceutical industry. The isotope development program of the Atomic Energy Commission is reviewed.

Advances in Pest Control Research. Vol. 3. Edited by R. L. METCALF. Interscience Publishers, Inc., 250 Fifth Ave., New York 1, N. Y., 1960. vii + 448 pp. 15.5 × 23 cm. Price $14.50.

This third volume of the series continues the excellent reporting in this special field. Volumes I and II were reviewed, respectively, in THIS JOURNAL, **47**, 230(1958), and **48**, 258(1959).

The Search for New Antibiotics. By G. F. GAUSE. Yale University Press, New Haven, Conn., 1960. x + 97 pp. 14 × 21.5 cm. Price $4.75.

This second volume in the Trends in Science program is based on a lecture in December 1959 by G. F. Gause, Director of the Institute of Antibiotics of the Academy of Medical Sciences, Moscow, U. S. S. R. Principles which are known and represent the scientific basis of the search for new antibiotics are given by Gause as: A general microbiological approach to the geography and ecology of microorganisms producing antibiotics; Early classification of microorganisms in the screening program; and Development of new test organisms for rapid detection of specific selective inhibition of some biochemical mechanisms, as illustrated by the search for potential anticancer products.

British National Formulary 1960. Alternative Edition. The British Medical Association and the Pharmaceutical Society of Great Britain. The Pharmaceutical Press, London, England, 1960. Obtainable in the U. S. from The Rittenhouse Bookstore, 1706 Rittenhouse Sq., Philadelphia, Pa. 292 pp. 10.5 × 16.5 cm. Price $1.75, interleaved $2.25.

This "alternative" edition of the B. N. F. 1960 contains much of the same information as does the standard edition; but the arrangement of the drug section in this edition is based on a pharmacological classification. These books are intended as guides in prescribing.

An Introduction to Pharmacology. By J. J. LEWIS. E. and S. Livingstone Ltd., Edinburgh and London, 1960. Exclusive U. S. agents, The Williams & Wilkins Co., 428 East Preston St., Baltimore 2, Md. xii + 826 pp. 14 × 21.5 cm. Price $11.

This book stresses the site, mode, and type of action of drugs, with attention to chemical structure and action relationships. It indicates the lack of knowledge of modes of action of some of the most widely used drugs and develops the text material to inspire further research. This is to be expected from an author who is still actively engaged in research in this field. The text material is presented in an interesting manner, omitting details available in more comprehensive publications. It is a good review and reference book.

Aids to Biochemistry. 5th ed. By S. P. DATTA and J. H. OTTAWAY. Baillière, Tindall and Cox, London, 1960. Exclusive U. S. agents, The Williams & Wilkins Co., 428 East Preston St., Baltimore 2, Md. viii + 266 pp. 10 × 16 cm. Price $3.75.

This compact book is based on the lecture course in biochemistry for preclinical medical students at University College, London.

Biochemistry of Steroids. By ERICH HEFTMANN and ERICH MOSETTIG. Reinhold Publishing Corp., 430 Park Ave., New York 22, N. Y., 1960. xi + 231 pp. 15 × 23 cm. Price $5.75 college, $6.90 trade.

This book is designed as a brief introduction to the biochemistry of steroids in which organic chemistry is kept as simple as possible. It is intended for students and for organic chemists in the pharmaceutical industry. Arranged for understanding of biogenetic relations, the chapter heads are: Cholesterol, Sterols, Vitamin D group, Steroid sapogenins and alkaloids, Cardiac glycosides, Bile acids, Progesterone, Corticosteroids, Androgens, and Estrogens. Each class is presented in monograph style. An extensive bibliography and an index are appended.

The Metabolism of Cardiac Glycosides. By S. E. WRIGHT. Charles C Thomas, 301-327 East Lawrence Ave., Springfield, Ill., 1960. viii + 86 pp. 15 × 23 cm. Price $4.75.

Much of the information in this book has been obtained since the development of sensitive methods of analysis, paper chromatographic techniques, and the availability of radioactive glycosides. The text material covers the chemical nature, structure, and pharmacological action of cardiac glycosides; their absorption, distribution, and excretion, and their metabolites.

Précis de Chimie Générale et de Chimie Minérale. Vol. 2. By L. DOMANGE. Masson et Cie., 120, boulevard Saint-Germain, Paris 6e, France, 1959. viii + 317 pp. 16 × 21 cm. Price 27 NF.

This second volume (in French) considers the elements of general chemistry (thermochemistry, equilibria, etc.), generalities of metals and their compounds, and descriptions of the metals and their compounds. Volume one was reviewed in THIS JOURNAL, **48**, 426(1959).

The Pharmaceutical Pocket Book. 17th ed. The Council of the Pharmaceutical Society of Great Britain. The Pharmaceutical Press, 17 Bloomsbury Square, London, England, 1960. Obtainable in the U. S. from The Rittenhouse Bookstore, 1706 Rittenhouse Square, Philadelphia, Pa. xii + 576 pp. 12 × 18.5 cm. Price 30s.

This edition, the seventeenth, of the book of general information for pharmacists is the first revision under the new (1959) Department of Pharmaceutical Sciences of the Pharmaceutical Society of Great Britain. The text material reflects the modern conditions in this field.

General Chemistry—Laboratory Manual. By GARTH L. LEE and HARRIS O. VAN ORDEN. W. B. Saunders Co., West Washington Square, Philadelphia 5, Pa., 1960. v + 217 pp. 22 × 28 cm. Paperbound. Price $3.75.

This manual of laboratory experiments is designed for students of general chemistry. Although no relating statement is made, the coverage of material is as broad as that in the textbook on the same subject by the authors of the manual.

Cahiers de Synthèse Organique. Vol. 6. Méthodes et Tableaux D'Application. Edited by LEON VELLUZ. Masson et Cie., 120, Boulevard Saint-Germain, Paris VI^e^, France, 1960. 417 pp. 16 × 22 cm. Price 120 NF.

This volume considers: Rearrangement of carbon structures, Functional migration between carbons, Functional migration between heteroatom and carbon, and Migration of carbon radicals between heteroatoms. Each section starts with a tabulation of chemical structures involved. This is followed by discussion of principles, mechanisms, applications, and reactions. A tabulation of functional groups and molecular arrangements created by degradation or transposition of compounds considered in vols. 5 and 6 is appended.

Documenta Geigy. Scientific Tables. 5th ed. with suppl. S. Karger, Basle, Switzerland, and New York, N. Y., 1959. Available in the U. S. from Albert J. Phiebig, P. O. Box 352, White Plains, N. Y. 547 pp. 16 × 23.5 cm. Price including postage and exchange $14.45.

This is the fifth edition of data compiled to provide the physician and researcher with basic scientific data in the fields of medicine, biology, chemistry, physics, and mathematics. Much useful information is concisely presented, much of it in tabulations. The table of usual and maximal doses is not sufficiently inclusive because it omits drugs that are in the N. F. Thus, it includes British drugs from the British Pharmacopoeia "or the British Pharmaceutical Codex"; however, the U. S. drugs are U. S. P. but not "or N. F." Since doses are given as they appear in the B. P. or B. P. C., U. S. P., P. I. (International Pharmacopoeia), etc., a complete comparison is not presented. A supplement covers constituents of living matter, metabolism, synthesis of cell constituents, and detoxication mechanisms. Included are nomograms for assessing body surface areas of children and adults. This is useful in the determination of therapeutic dosage.

Encyclopaedia of Microscopic Stains. By EDWARD GURR. The Williams & Wilkins Co., 428 East Preston St., Baltimore 2, Md., 1960. xi + 498 pp. 15 × 24.5 cm. Price $18.50.

This is a reference book and a laboratory guide on the applications, structures, composition, molecular weights, and properties of most of the important dyes and other substances used for staining microscopic tissue preparations. The major portion of the book (421 pages) is an alphabetical monograph arrangement of stains, indicators, etc. The appended general index is helpful.

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